THE EFFECT OF IMMUNIZATION ON DEFENSE MECHANISMS AGAINST INFECTIOUS DISEASE IN IRRADIATED ANIMALS

by

Eugene Hafen Perkins

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Eugene Hafen Perkins

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INTRODUCTION

It has been thoroughly established that x-irradiation results in increased susceptibility of different mammalian species to a variety of infectious agents. Bacteriological investigations on both naturally occurring and experimentally induced infections indicate that host resistance is markedly depressed following irradiation.

Active immunization is the recognized classical procedure for enhancement of host resistance. It seems apparent therefore, that active immunization prior to irradiation might afford protection to those animals in which morbidity and mortality is attributable to overwhelming infection.

Little is known concerning the role specific immunization plays on each of a wide variety of biological mechanisms which determine the degrees of susceptibility or resistance of the mammalian host to infection. Certain specific factors of immunity have been shown to be impaired by x-irradiation and it is this phenomenon of differential sensitivity of resistance mechanisms that makes this investigation of basic interest.

In the irradiated animal opportunity is provided to study certain contributing roles of specific resistance mechanisms independent of each other. Utilizing immunization for enhancement of specific resistance mechanisms and selectively inducing suppression of certain of these mechanisms by irradiation affords opportunity to assess the role each plays in the overall defense of the host. Consideration of "isolated

systems" which function as protecting agencies and the role immunization plays in augmenting such protection can be studied quantitatively. The studies to be reported have been carried out to gain insight into the fundamental factors and mechanisms concerned with specific immunity, to more clearly establish their relative significance and to determine if the depressed resistance of irradiated animals can be related to these factors.

REVIEW OF THE LITERATURE

I. ANTIBACTERIAL IMMUNITY

Resistance may be defined as the sum of all forces with which the mammalian host is endowed to oppose noxious influences that may disturb its natural economy. Natural resistance has been defined (Sabin, 1952) "to designate a refractory state which does not depend on immunologic processes resulting from previous exposure of the host to a particular infectious agent or its component antigens." The term "immunity" is reserved to describe multiple physiological changes that take place within the host to specifically enhance the fluctuating state of resistance. Immunity is relative rather than absolute and differs from natural resistance in that it is quantitatively more extensive, strictly specific, and is acquired. In the present discussion one aspect of enhanced host resistance, antibacterial as opposed to antitoxic immunity, will be treated. The discussion includes various mechanisms which may be accelerated such as defense against the act of invasion, inhibition of reproductive activity of the parasitic organisms, prevention of progressive infection and actual destruction of the invading organism independent of action against specific toxins.

Attempts to determine the nature of antibacterial immunity and resistance to infection too often have been attempts to reduce the mechanisms of resistance to terms of a single factor. Thus, only after an extended epoch of controversy between two major schools each seeking to explain resistance in terms of a single factor, namely humoral factors in one instance and body cells in the other, has the dependence of each of these factors on the other, and the fallacy of attempting to eliminate

either come to be recognized. Nevertheless, critical investigation is still necessary, not to eliminate one thesis in terms of the other, but to more clearly establish the significance, preferably in quantitative terms, of the role played by each in resistance to infectious disease.

A. The Role of Antibody in Antibacterial Immunity.

An important consideration in the study of antibacterial resistance, is the question of the functional role of antibody (agglutinins, precipitins, opsonins, lysins, sensitizins, etc.), that is, the contribution of agglutination, precipitation, bacteriolysis, etc., as phenomena contributing to the protection of the body against infection. What is the relationship between the presence or production of these specific substances and acquired resistance? Specific antibacterial antibodies may be used to gain information concerning the nature of the bacterial species giving rise to the infection; however, the common procedure of defining resistance in terms of antibody level, that is, a rise in titer correlated with increased resistance, is not substantiated by experimental evidence.

1. Agglutination of bacteria. In early studies Salimbeni (1897), working with cholera vibrio, came to the conclusion that bacterial agglutination does not take place within the animal's body. He was unable to demonstrate in vivo agglutination in the peritoneal fluid of guinea pigs, although this fluid caused immediate agglutination when mixed with vibrios in the test tube. Similarly, this worker found that in horses, goats, and guinea pigs with high titers of antibody, induced by active or passive immunization, that agglutination did not take

place when the microorganisms were injected subcutaneously and small amounts of the exudate were removed at frequent intervals and viewed either microscopically or macroscopically.

The first extensive experimental investigation, however, on agglutination in vivo was made by Bull (1915a). He observed that anti-pneumococcus serum caused agglutination of pneumococci in vitro and in vivo. Antiserum, injected into the circulation of rabbits with pneumococcus bacteremia, brought about instantaneous clumping of the diplococci in vivo and removal by the liver, spleen, and lungs. Normal rabbit blood was devoid of agglutinating power. The protection afforded by the immune serum was found to be specific for the type organisms. Bull concluded that "agglutination is not merely an incidental phenomenon, but constitutes an essential process in association with phagocytosis in the protection of the rabbit against pneumococcus infection."

In succeeding work, Bull observed (1915b) that typhoid bacilli are agglutinated promptly in the circulating blood of normal rabbits (the rabbit may be regarded as possessing a high degree of natural resistance for typhoid bacilli). The clumps of bacilli were observed to be phagocytized, following which process these organisms were assumed to be digested and destroyed by the phagocytes.

The same year Bull (1915c) noted that typhoid bacilli agglutinated spontaneously in the circulation of the normal rabbit even in cases in which undiluted serum gave a negative result in vitro. Dysentery bacilli of the Shiga type did not agglutinate in the blood stream of the normal rabbit, but small quantities of antiserum injected into the circulation caused immediate agglutination, while all strains of Flexner bacilli underwent spontaneous agglutination. Agglutination of the bacteria

within the blood of the infected animal was followed by a rapid removal of the bacteria from the circulation by phagocytosis while those bacteria not agglutinated remained in the circulation and produced a progressive septicemia. "Hence the agglutinins seem to play the decisive part in at least certain instances of bacterial infections."

A concluding effort by Bull (1916) on this problem revealed that pneumococci, dysentery bacilli of the Shiga type, and Bacillus mucosus capsulatus (Friedlander's bacillus) were agglutinated immediately when injected into the circulation of actively immunized rabbits. Staphylococcus aureus and S. epidermidis, colon bacilli, meningococci, gonococci, and nonvirulent pneumococci agglutinated in the circulation of normal rabbits. Although Bull stated that "the degree of agglutination and opsonization of bacteria within the animal's body is inversely parallel to the infectiousness of the bacteria for the host," he was careful to note that exceptions might be found to this rule.

That agglutination of bacteria might be a mechanical aid to phagocytosis had previously been suggested by Besredka (1901), who observed that guinea pigs survived when challenged intraperitoneally with typhoid bacilli which had previously been mixed with serum from normal animals. The resistance was directly proportional to the degree of previous agglutination of the bacilli; the organisms were rapidly phagocytized when in the form of "clumps."

Ten Broeck (1917) injected virulent hog-cholera bacilli into normal and immunized rabbits and observed rapid clumping and disappearance from the circulation. He was unable to explain the role of these agglutinating antibodies in relation to immunity, since agglutinin titers could not be correlated with resistance.

Rich and McKee (1934), in efforts to establish the significance of humoral alterations in antibacterial infections, independent of phagocytes, injected benzol to reduce the leucocyte count and then infected immunized animals intradermally with virulent type I pneumococci. It was found in these immunized leucopenic animals that the bacteria proliferated, adhered to each other and apparently to the tissues as well, and were thus held fixed, in the absence of microscopically detectable inflammatory exudate or thrombosis of lymphatics for hours after non-immune controls had died of septicemia. The workers deduced that since the phenomenon of immobilization occurs in passive as well as in actively immunized animals, it is the antibody content of the fluid which is primarily responsible for the prevention of the prompt spread of the bacteria; however, in the absence of leukocytes, the growth of the immobilized bacteria proceeds until the bacteria eventually penetrate the blood stream and the animal dies with a septicemia even though its plasma antibody is able to passively protect non-immune animals possessing leukocytes.

Angevine (1936) observed that virulent streptococci injected into the skin of normal rabbits multiply actively, resist phagocytosis, invade the tissue widely, enter adjacent and distant lymph nodes and in some instances were distributed by the blood stream to internal organs. After immunization, virulent streptococci were more readily ingested by phagocytes, remained more sharply localized, were rapidly destroyed, failed to pass the nearest lymph nodes, and did not enter the blood stream. Histological sections of lesions showed more phagocytosis in immunized than in normal rabbits.

Wood (1941) noted that the principal effect of antipneumococcus

serum is to cause immobilization of the pneumococci in the advancing edema zone in lobar pneumonia. Antibodies were demonstrated in the alveoli within 10 minutes after treatment and, apparently, immobilized by agglutination, the organisms were overtaken and destroyed by phagocytes.

Similarly, Cannon and Pacheco (1930) Catron (1935), Cannon and Hartley (1938), and Pacheco (1932) have injected various microorganisms into tissue of immunized and normal animals and observed early and marked agglutination followed by active phagocytosis. Interaction between antibodies and bacteria materially assisted in prompt bacterial localization.

Agglutination per se has no detrimental effect on microorganisms (Oldfelt, 1942). It would appear that the functional significance of agglutinins in body defense, whether occurring normally or as a result of specific immunization, is to facilitate the disposal of organisms which have gained parenteral entrance.

2. Bacterial precipitins. Although the infected host may develop precipitins due to stimulation by the infecting organism, and the detection of these may be of diagnostic value, no one has conclusively demonstrated that a precipitate is actually formed in the blood (Cannon, 1940). There is, however, indirect evidence that this may occur. While the protective action of precipitins in antitoxic immunity is well established, the role played by precipitins, if any, in antibacterial resistance still requires experimental clarification.

Evidence for the occurrence of precipitation in the tissue has been furnished by Opie (1923, 1924a, 1924b). He injected crystalline egg albumin into the skin of normal rabbits and observed that it quickly diffused from the site of injection, but that with each succeeding

injection, at intervals of a few days, more was retained at the injection site. He concluded that the local retention of antigen was due to union of the precipitin and the antigen. Cromwell and Centeno (1929) noted that after intravenous injection of crystalline egg albumin into immunized rabbits, that vacuolated mononuclear cells appeared in the peripherial blood. In in vitro experiments, these authors observed similar vacuolation of cells when leukocytes were added to a mixture of egg albumin and antisera, but failed to observe vaccuolation when normal sera was substituted for the antisera. They concluded that the vacuoles were associated with phagocytosis and the digestion of specific precipitate.

On the other hand, other workers (Grove, 1932; Seibert, 1932 and Kahn, 1933) have failed to observe any relationship between the precipitative strength of a serum and the intensity of the cutaneous reaction to its antigen.

Rothbard (1945) has even recorded a diminution of the bacteriostatic effect of leukocytes of blood for Group A streptococci when specific precipitates are present by blockade of these cells. This investigation was carried out in vitro. A comparable condition in vivo would necessitate an absolute blockade of the reticulo-endothelial system, a condition seldom, if ever, achieved under natural or experimental conditions; consequently, it would seem that precipitates may not play a significant role in impairing resistance under normal conditions.

Nevertheless, since it has been established that the predominant factor in the infectivity (ability to establish an infection) of the pneumococcus is the capsular polysaccharide, investigations of the precipitin reaction with S.S.S. (specific soluble substance) have also

been carried out. Friedlander, Sobotka and Banzhaf(1928), Heidelberger, Sia and Kendall (1930) and Zozaya, Boyer and Clark (1930) have shown by different methods that a positive relation may exist between the precipitation power of a serum and protective power. Felton (1930a, 1930b) concluded from extensive investigations that, of the *in vitro* methods available, the precipitin reaction was the one of choice and Smith (1932) states that the precipitation value is a true index to the protective power of anti-pneumococcal serum.

3. *Bacterial lysins*. It has been conclusively demonstrated that certain antibody, independent of cellular activity, can destroy certain bacteria. Most often this is mediated by the action of complement; on the other hand, there are certain instances where antibody *per se* exerts a lytic effect. What is its role in protection or resistance?

Pfeiffer and Issaeff (1894) showed that when cholera vibrios are introduced into the peritoneum of animals immunized against vibrios that the organisms undergo extracellular lysis. This action is brought about by specific antibody (immune-body, sensitizer, amboceptor) in conjunction with complement. However, Bordet (1895) early assured himself that when cholera bacilli were injected into the circulation of an actively immunized animal they were primarily phagocytized rather than destroyed extracellularly.

This "immune" type of bacteriolysis or bactericidal action is seen in a relatively small proportion of organisms and a great many species are not susceptible to this action. Raffel (1953) suggests that the cholera vibrio, some of the Gram negative enteric bacilli (*Salmonella typhi*, *Pseudomonas aeruginosa*), probably *Brucella* species and *H. influenzae* are susceptible to *in vivo* lysis. Therefore, the importance of this

phenomenon in specifically induced resistance would seem to be subordinate to resistance induced by types of antibody which lead to phagocytosis of corresponding bacteria.

Furthermore, Bull (1915b) demonstrated that typhoid bacilli are promptly agglutinated in the circulating blood of normal rabbits. The clumps are taken up by phagocytes where they are presumably digested and destroyed. Thus, "destruction of typhoid bacilli intra vitam is brought about by an entirely different process than is the destruction by serum and whole blood in vitro. While the latter is caused by bacteriolysis, the former results from agglutination and intraphagocytic digestion."

4. Bacterial opsonins. The importance of plasma or serum in furthering phagocytosis became evident when Denys and LeClef (1895) showed that in the presence of antiserum leukocytes of normal animals ingested streptococci as rapidly as did those of an immunized animal.

Leishman (1902) described a method of counting the number of organisms ingested by leukocytes in vitro. This worker confirmed the results of Denys and LeClef (1895) and his work, in turn, was confirmed by Wright and Douglas (1903, 1904) who introduced the terms "opsonin" and "opsonic index." Neufeld and Rimpau, (1905) showed that the increase of the phagocytosis-promoting action of serum from immunized animals was due to a thermostable antibody which they called "bacteriotropin."

However, these antibodies present two potential mechanisms of action: a) opsonic effect may be produced by antibody alone, and b) the opsonic effect may be due to the interaction of complement along with antibody. The term "tropin" has been applied to the former while the latter is designated as opsonic immune body, immune opsonin or bacteriotropin.

Thus, a bacteriotropin is a thermostable specific antibody which, by itself, produced the essential change in a bacterium, resulting in susceptibility to phagocytosis. The opsonic effect of an "immune" serum may, however, in addition be due to its content of antibody which does not by itself opsonize the bacteria, but which acts by leading to the union of complement. The relative significance of these two possible modes of action in promoting phagocytosis remains to be investigated. The important practical consideration that phagocytosis can be enhanced by the action of serum substances on microorganisms is well established.

Manwaring and Coe (1916) demonstrated the value of antibody as an aid in in vitro hepatic clearance. By carefully washing livers free from blood and then perfusing the organs with virulent pneumococci they found that whereas virulent pneumococci treated with immune serum were quantitatively removed by the cells of the liver, these organisms suspended in Ringer's solution or in normal serum were not removed by the liver. As smears and histological preparation made from the perfused liver showed numerous pneumococci adherent to capillary endothelium with few, if any agglutinated masses and little or no endothelial phagocytosis, they attributed the action to a substance which for lack of a better term they called "endothelial opsonin."

Wright (1927) in a monumental work on experimental pneumococcal septicemia and antipneumococcal immunity, concluded that the outstanding effect of immunization with type I pneumococci is the enhancement of activity of the body fluids favoring phagocytosis. The possible existence of a residual "cellular immunity" was not excluded by Wright. Agglutinins, opsonins, precipitins and complement fixing antibodies as measured by in vitro tests did not appear to be essential as such.

Nevertheless, antibody transferred passively to normal animals was protective.

Robertson and Van Sant (1939) reported a comparative study of phagocytosis and digestion by macrophages and polymorphonuclear leukocytes in normal and immunized dogs. It was found that while the macrophages showed a somewhat greater phagocytic activity than the polymorphonuclear leukocytes, their ability to digest pneumococci was much more pronounced in the presence of opsonic fluid of relatively low concentration. Both normal and "immune" cells were found to be dependent on opsonins for the phagocytic activity.

Kerby et al. (1950) observed that animals (rabbits and dogs) which survived an induced bacteremia (Klebsiella pneumoniae) were capable of clearing, at an accelerated rate, bacteria from the circulating blood following a second exposure (infusion). The increased removal could also be demonstrated quantitatively following the administration of plasma from a dog previously given injections of the same organism, while normal dog plasma failed to significantly affect the removal.

Thus, an important aspect in relation to the resistance produced by immunization is the development of thermostable specific antibodies which promote phagocytosis. The action may be a direct one on the organism or assisted through the medium of combined complement. It may be stated as a general theory that antisera generated by the injection of organisms which act as antigens will have opsonic properties.

5. Lack of correlation between antibody titer and specific resistance. The resistance of animals inoculated with infectious agents often has been ascribed to the antibodies which the inoculum induces. Nevertheless, one cannot always correlate antibody titer and resistance to infection.

As early as 1899, Gengou (1899) reported that human serum may contain large amounts of agglutinins for attenuated anthrax bacilli, yet man is far from being immune to the disease. "In certain cases agglutinins for microorganisms may apparently be entirely absent and yet the animal enjoys an immunity."

Ten Broeck (1917) observed that rabbits may show a high agglutination titer to hog cholera bacillus and have no immunity and, on the other hand, resistant animals may show comparatively low agglutination titers.

Topley (1929) demonstrated that the "O" antigen is the single significant factor in producing specific immunity against Salmonella aertrycke. However, of animals which showed significant resistance (as measured by mortality) only 21 of 240 mice showed an "O" titer as high as 1:20. Since dilutions were not carried out below this level, one can only speculate concerning the proportion of mice which showed no titer. Thus, here is a demonstration of immunity without detectable or very low levels of protective antibody.

Working with S. aertrycke, Schutze (1930) obtained "O" titers of 1:100 or higher in 46 mice, or 1:200 or higher in 17 mice and 1:400 in 5 mice. However, although he succeeded in obtaining higher titers than Topley, very little correlation was observed between the average somatic agglutinin titer of a group and its resistance to infection.

Wadsworth and Kirkbride (1917) in experiments to produce an anti-pneumococcal serum as a therapeutic agent, immunized horses against Type I pneumococci. One-tenth ml of this serum protected mice against 0.5 ml of a virulent culture, 0.000001 ml (0.1 of 10^{-6}) of which killed mice in less than 40 hours. Serum produced against type II organisms gave similar results. However, agglutination reactions indicated an

inverse relation between the agglutinating power and the protective value of the sera, and in each type the horse serum with a lower protective index gave a higher agglutination titer.

Wood et al. (1951) demonstrated the importance of phagocytes in induced bacteremia with pneumococci and Friedlander bacilli. Intravascular phagocytosis could be seen to take place within fifteen minutes after inoculation, in spite of the fact that the serum of the rabbits contained no detectable opsonins.

Cecil and Blake (1920a, 1920b) pointed out the lack of correlation between protective substances present in serum and the ability of monkeys to withstand pneumococcal (pneumonia) infections.

Attempts to evaluate past efforts to determine the relation between antibody titer and protection, must take into consideration the fact, that antisera prepared by the injection of whole organisms, may contain non-agglutinating, non-precipitating, or tissue bound protective antibodies directed against invading organisms in the absence of demonstrable circulating antibody. Kabat, et al. (1945) documented a failure to find any correlation between agglutinin content and the protective capacity for mice or type I antimeningococcal sera derived from horses, rabbits, chickens and humans convalescent from the corresponding infection. Combining but non-precipitating antibody was prepared in horses by Heidelberger et al. (1940) using chicken egg albumen as antigen. Morgan and Schutze (1946) immunized humans against Sh. shiga and S. typhi bacilli and proved, by use of the Coombs test, the presence of non-agglutinating antibody. Freter (1950) has demonstrated variation in agglutinating and protective capacities of antibodies at various times following immunization. Hayes, Dougherty and Gebhardt (1951) showed that the

lymphocytes of immunized animals contained surface agglutinating factors even though separated from serum. Also, specific antibodies readily become fixed to tissue cells (i.e., as in anaphylaxis). Therefore, the absence of circulating antibody cannot be accepted as definitive evidence that specific protective antibodies may not be involved in the observed resistance of immunized animals.

To summarize the role played by humoral factors in antibacterial immunity, it must be concluded that most evidence suggests that the humoral part of the process is to facilitate, that is, act as an aid to phagocytosis. It has been shown by passive immunization that specific antibodies play an important role in antibacterial resistance, but the exact nature and functional role of antibody in resistance is frequently expressed in an unrealistic manner.

B. The Importance of Cellular Mechanisms in Antibacterial Immunity

The cellular aspect of immunity, first described by Metchnikoff (1905), has been accorded increased significance, and has become an established and accepted current principle. According to present day concepts the phagocytic system represents a functional unit composed of widely dispersed and morphologically distinct cell types (polymorphonuclear and mononuclear leukocytes of the circulation and the cells of the reticulo-endothelial system). "The circulating leukocytes and reticulo-endothelial cells appear to supplement one another in destroying the bacteria that have gained access to the circulation . . . (and) impaired function of one system is compensated by the other mechanism" (Wood *et al.*, 1951). The significance of these "cellular mechanisms"

employed by the host to localize infection or to free the blood stream of bacteria as illustrated in the preceeding section, has been recognized as the most important element in antibacterial resistance (Gay et al., 1935). Furthermore, the concept has long been held that humoral elements supplement but in no way minimize the predominant importance of phagocytosis in resistance. Strong evidence (Gay and Morrison, 1923; Gay and Clark, 1926; Linton, 1928; Freedlander and Tooney, 1928; Bull, 1915; Manwaring and Coe, 1916; Hopkins and Parker, 1918; Sullivan et al., 1934; Reichel, 1939 and Bennett and Beessor, 1954) exists to suggest that resistance depends on maintaining, at a high level, an efficient clearing mechanism (active phagocytosis) which insures prompt removal of any bacteria which gain access to the host; further, that the increased antibacterial resistance that an actively immunized animal enjoys is in part dependent on the increased efficiency of phagocytic function. The importance of cellular factors in resistance to infectious disease has been discussed by Bordet (1939) and may be summarized as follows:

a. A parallelism exists between the efficiency of phagocytosis and resistance to infection.

b. The bactericidal power attributable to humoral factors of the blood cannot alone account for destruction of pathogenic microorganisms. It is intracellular digestion that usually brings about destruction of the parasites.

c. The virulence of microorganisms is often associated with antiphagocytic properties.

d. The sequence of events in a disease process is usually less serious when inoculation of organisms is made at a site abundant in phagocytes.

e. All factors which interfere with phagocytosis diminish the resistance of animals to infection.

f. The major significance of phagocytosis is shown by the fact that a favorable course in a disease process is usually accompanied by an increase of leukocytes. In the immunized animal, following infection, leukocytosis is pronounced and is maintained for a long period of time.

1. Variation in phagocytic activity. Variations in the inherent phagocytic power of leukocytes has been suggested by Glynn and Cox (1909). They investigated the phagocytic ability of leukocytes of three normal persons, one case of mild staphylococcus infection, and several miscellaneous cases. Their results demonstrated variations in phagocytic power on the part of leukocytes from different persons and from the same person at different times.

Madsen and Wulff (1919) in presenting results on the influence of temperature on phagocytosis, have shown that the maximum phagocytic activity of leukocytes occurs at the temperature of the person or animal from which they are taken. For example, in man at 37°C, in guinea pigs at 39°C, and in the cock and the pigeon at 41°C.

Rosenow (1906) was among the first to study variations of phagocytic ability. His results indicated that leukocytes from pneumococcus pneumonia patients have a greater inherent phagocytic capacity than did phagocytes from healthy individuals. The preponderance of younger forms which occur in response to the infection was suggested as the basis for this difference. Not only did Rosenow find that leukocytes from pneumonia patients would take up pneumococci more easily than did normal leukocytes under the same conditions (that is, using the same serum) but also, that such leukocytes would take up pneumococci that would resist

phagocytosis by the leukocytes from normal blood. He noted that similarly, leukocytes from cases of acute appendicitis and puerperal infection also appeared to be endowed with increased powers of phagocytosis.

Park and Biggs (1907) reported that leukocytes from different normal persons may vary in their phagocytic power for staphylococci.

Hektoen (1911) reviewed the subject of phagocytosis; he stressed the need, in comparative studies of giving due attention to the matter of having equal numbers of phagocytes in the mixtures made for the comparison of the phagocytic power of leukocytes from different sources. This criticism undoubtedly applies to some of the early observations bearing on this question. In addition, some workers have failed to report if the studies carried out have utilized the same serum; thus, the problem of humoral antibody and the role it is playing in the observed differences in phagocytic function is often difficult to assess.

Some studies have been made on the relative phagocytic power of the different types of polymorphonuclear leukocytes classified according to the Arneth classification. Results have varied. Briscoe (1907) and Pottenger (1909) concluded that leukocytes of Arneth's classes II and III have greatest phagocytic activity. As phagocytable bacteria they used staphylococci and tubercle bacilli. On the other hand, Kaplin (1907) found that, so far as staphylococci are concerned, the cells with one lobed nucleus were more actively phagocytic and those with four lobes, the least active. Briscoe (1907) also noted that the cells found in the alveoli of the lung and mononuclear cells found in the peritoneal cavity showed marked phagocytic capacity. In vitro this activity was distinctly less than the polymorphonuclear leukocytes, but in vivo it was a great or greater.

Tunnicliff (1910) reported that at birth the phagocytic activity of leukocytes is below that of normal adults with regard to streptococci, pneumococci, and staphylococci. The adult standard seems to be reached during the third year of life. The low phagocytic power of leukocytes in infancy as compared with later years would seem to be inherent in the leukocytes as this difference exists in phagocytic mixtures prepared with the serum of adults as well as with the serum of infants.

Tunnicliff (1911a) determined also that the leukocytes of induced exudates of guinea pigs and hogs had greater phagocytic activity than blood leukocytes. Using suspensions containing the same number of carefully washed polymorphonuclear leukocytes, the leukocytes of the exudate not only took up a larger number of leukocytes engaged in phagocytosis than in the case of leukocytes obtained from the blood.

Bayly (1908) observed a reduction in the phagocytic power of leukocytes for tubercle bacilli as a result of fatigue.

Potter (1907) reported that differences exist in the phagocytic activity of leukocytes. He found that leukocytes from bacterially infected patients did not always show as extensive phagocytic activity as the cells of supposedly normal individuals. During recovery from some infections (staphylococcus, streptococcus) phagocytic activity surpassed the leukocytes of supposedly normal individuals, and during convalescence their phagocytic activity fell to or below that of normal leukocytes.

2. Effect of immunization on cellular elements. Metchnikoff (1905) in early efforts postulated a "training" or "education" of cells in immunity. It remains to be determined whether the phagocytes of actively immunized animals can respond specifically by more rapid mobilization, increased

rate of phagocytosis and a greater or more rapid digestive capacity than phagocytes of normal animals. Approaches to these problems have received little attention in the past and are currently under more active investigation.

Among early publications which did not support Metchnikoff's ideas were papers by Tunnicliff (1911b) and by Denys and Leclef (1895).

Tunnicliff (1911b) found that there is an increase in the phagocytic power of leukocytes in favorable cases of pneumonia, both before and after the "crisis." This variation was nonspecific and could be demonstrated also with streptococci and staphylococci. Similar nonspecific variations were observed in severe cases of scarlet fever.

Denys and Leclef (1895) reported that in the presence of antiserum, leukocytes of normal animals ingested streptococci as rapidly as did those of an immune animal. On the other hand, in the presence of normal serum there was absence of phagocytosis, no difference was noted between the leukocytes of the normal animal and those of the immune animal.

A swing away from these concepts and toward Metchnikoff's original ideas began with the work of Lurie (1939). This investigator demonstrated that mononuclear cells derived from actively tuberculous or vaccinated guinea pigs and rabbits, exhibit significantly greater *in vitro* phagocytic capacity for carbon particles, staphylococci and tubercle bacilli than mononuclears obtained from normal animals. This difference could not be attributed to undectable antibody as significant differences were obtained in the presence of the serum from both normal and sensitized animals.

Furthermore, Lurie (1942) observed that mononuclear phagocytes of immunized animals that had ingested tubercle bacilli *in vitro* in the

presence of either normal or "immune" serum inhibited the multiplication of the microorganism in their cytoplasm to a much greater extent than did cells of normal animals that had ingested the bacteria in the same medium and had grown in a similar environment. The presence of "immune" serum during the in vitro ingestion of tubercle bacilli did not regularly endow them with increased bacteriostatic properties. With in vivo studies it was again demonstrated that phagocytes of immunized animals, that had ingested tubercle bacilli and had subsequently been transplanted and grown in the environment of a normal animal, continued to inhibit multiplication of the microorganism in the absence of immune bodies, Lurie concluded that active tuberculosis confers on the mononuclear phagocytes themselves increased bacteriostatic properties independent of immune body fluids.

Similarly, Suter (1953) has shown in vitro that multiplication of attenuated and virulent tubercle bacilli occurs readily within phagocytic cells of normal guinea pigs and rabbits, especially in monocytes and macrophages, but that the growth process of these organisms is greatly retarded or completely inhibited within phagocytes from animals vaccinated with BCG. Moreover, the serum of vaccinated animals added to this system, did not inhibit multiplication of tubercle bacilli within monocytes derived from normal animals. This inhibition appears to be independent of the presence of humoral factors in the culture medium, a fact which lends support to the assumption that as a result of immunization the phagocytes themselves become endowed with the ability to interfere with the intracellular multiplication of tubercle bacilli. These observations with tubercle bacilli were later supported by experiments of Raffel (1955) who found inhibition of multiplication of tubercle bacilli in macrophages

obtained from BCG vaccinated guinea pigs. Pomales-Lebron and Stinebring (1957), have reported a similar inhibitory effect of macrophages obtained from animals immunized against Brucella abortus. However, Mackaness (1954) could find no significant differences in the intracellular activity of phagocytes derived from normal or immunized animals.

Teale (1935) immunized (first with dead, then attenuated, then living virulent cultures) rabbits against highly virulent streptococci. The rabbits thus immunized were injected intravenously with large numbers of virulent organisms and were able to rapidly clear the circulation and prevent reinvasion by the actively growing virulent bacteria. Blood failed to show germicidal or serum agglutinative power. Protective power was tested by passive immunization of mice with 0.5 ml of sera, then inoculating, 18 hours later, with about 5 MLD. Under these conditions no protection was noted. Similar experiments were conducted with B. anthracis, and Diplococcus pneumoniae, type III. Teale concluded that the chief factor in immunity is the "state of the tissues" with regard to the infecting bacteria in question. The "state of the tissues" concept was closely related to phagocytic cells.

Street (1942) reported that cells obtained from pleural exudates from immunized rabbits (pneumococcus type II, R) and transferred to normal animals, would not protect against infection with pneumococcus type I. However, normal rabbit cells resuspended in supernate from the pleural exudate of pneumococcus type II R immune animals, produced a high degree of protection against pneumococcus type I. These data are summarized in the following table, adapted from Street's paper.

Treatment	No. of animals	Challenge dose Pneumo. I MLD	Mortality ratio
Given immune cells in normal supernate intrapleurally before infection.	3	100	2/3*
Given normal cells in immune supernate intrapleurally before infection.	3	100	0/3
Given immune supernate intrapleurally before infection.	3	100	3/3
None	3	1	3/3

*Dead over total

Contrary to Street's conclusions, that is, that there is no evidence that cells from an immunized animal are by themselves specifically attuned for destruction of pneumococci, it seems that suggestive evidence is provided from this limited study that "immune" cells may have completely protected the animals had the infective dose been of lesser magnitude. Where 1 MLD produced 100% mortality in untreated animals, treatment with immune cells protected 1 of the 3 animals when a hundred-fold greater challenge was administered. Equally significant are the dramatic effects of the uselessness of antibody without the presence of adequate numbers of cells.

From the reviewed findings it appears that Metchnikoff's concept that macrophages of immunized animals exhibit a superior response to infectious agents, independent of humoral antibody, deserves further consideration.

C. Serum Bactericidal Activity and It's Role in Antibacterial Immunity

A stimulating literature has accumulated reporting the existence of the bactericidal activity of serum, blood and plasma. In early efforts Nuttall (1888) demonstrated that defibrinated blood was destructive for certain species of bacteria and this bactericidal property was lost upon heating at 52-56°C for ½ to 1 hour. The following year Büchner (1889a, 1889b) reported that cell free serum possessed bactericidal activity and that this activity was lost when serum was heated for 55°C for 1 hour. Among the earlier workers who attempted to elucidate the role of complement in bactericidal activity of blood and serum was Liefman and Slutzer (1910), Braun (1911) and Boehnche (1912).

In 1899 Moxter (1899) observed that complement required an additional normal serum component to manifest bactericidal activity. Steinhardt (1905) found that bactericidal action for both typhoid and dysentery bacilli was removed from normal serum by dead cultures of either organism and ascribed the result to the action of a common, naturally occurring "immune" body. Among the early authors who concluded that the bactericidal activity of normal serum was due to the presence of complement and a nonspecific "natural", "sensitizing" or "intermediate" antibody were Muir (1909), Gordon and Wormall (1928), Gordon and Carter (1932), Gordon (1933), Gordon and Hoyle (1936), and Mackie and Finkelstein (1931, 1932). The investigations of many workers have since confirmed and extended these reports.

Behring (1888) demonstrated that rat serum contained a thermostable bactericidin for Bacillus anthracis. Pirenne (1904) noted that

the thermostable rat serum, anthracide, proved bactericidal for various gram positive bacteria but was inactive against gram negative species; hence two bactericidal mechanisms exist, one inactivated at 55°C and the other stable at this temperature. More recently Dònaldson (1954), Myrvik and Weiser (1955), Fishman and Shechmeister (1955), Wood and Ono (1958) and Ekstedt (1956a, 1956b) have reported a relatively heat stable substance in normal serum which is active against gram positive bacteria.

Pillmer and co-workers (1954) isolated and characterized a protein (properdin) from normal human serum which required complement and Mg^{++} to exhibit bactericidal activity. Properdin exhibits bactericidal activity only against gram negative bacteria, with the exception of B. subtilis, and among the gram negative organisms many strains are resistant to its activity and other gram negative strains are susceptible to serum deficient in properdin. Skarnes and Watson (1957) in a recent review focused attention on the strong similarities between properdin and normal antibody and concluded that these are likely the same substance.

A problem still under active investigation is the role of these bactericidal substances in natural and acquired resistance to infectious disease. In 1922 Ledingham (1922) cautioned against ascribing too much significance to the various bactericidal factors described in the literature, particularly since most of these factors have been tested in vitro only. Skarnes and Watson (1957) states that "this caution is even more warranted today" and Wilson and Miles (1955) recognizing the difficulty in trying to evaluate these reactions as protective mechanisms concluded from the evidence available that increase in the

bactericidal power would seem, for the most part, too slight or too transient in their effect on resistance to be worth exploiting as prophylactics against infection.

It is true that bactericidal activity might augment or enhance other host defense mechanisms and indeed may represent one facet of the whole defense system. However, at least in specifically acquired resistance, this action might be negligible since it has been shown that whereas small amounts of heated "immune" serum added to normal serum enhanced bactericidal activity, the addition of larger amounts of specific "immune" serum caused a reversal or complete loss of normal serum bactericidal activity (Neisser and Wechsberg, 1901; Maaloe, 1946; Wardlaw and Pillemer, 1956).

II. EFFECT OF X-IRRADIATION ON SPECIFIC RESISTANCE MECHANISMS.

Postirradiation enhanced susceptibility to infection is recognized as one of the major causes of death among animals exposed to ionizing radiation in the lethal to mid-lethal range (Talmage, 1955; Raffel, 1956). Evidence available suggests that the increased susceptibility can be attributed to the deleterious effects of x-irradiation on the depression of specific host defense mechanisms (Taliaferro and Taliaferro, 1951). Suppression or marked delay of antibody formation, impairment of cellular response and loss of normal serum bactericidal activity are among the most prominently implicated mechanisms.

A. Effect of Irradiation on Antibody and Antibody Formation

Benjamin and Sluka (1908) were among the first to study the effect of x-rays on antibody production. Using beef serum as antigen they observed that irradiation delayed the rate of formation and lowered precipitin titers in dogs. No inhibition of precipitins was observed by these investigators if the animals were irradiated 4 days following antigen injection or at the height of antibody production. The following year Lawen(1909) reported inhibition of formation of specific agglutinins and lysins in rabbits following x-irradiation. Frankel and Schillig (1913) observed decreased agglutinin titers when animals were irradiated prior to injection with typhoid bacilli. Similarly Simonds and Jones (1915) exposed rabbits to small daily doses of x-rays and, following a single intraperitoneal injection of killed typhoid bacilli, noted that the formation of agglutinins were appreciably lowered.

Von Heinrich (1913) reported that x-irradiation inhibited anaphylaxis when animals were exposed to x-rays shortly after antigen injection. However these same animals were susceptible to severe anaphylactic shock six weeks later. This investigator suggested that irradiation interfered temporarily with the production of antibody upon which anaphylaxis depends.

Hektoen (1915) studied hemolysin response in rabbits prior to and during administration of daily small doses of irradiation. He observed that when antigen was given prior to first exposure, hemolysin production was not appreciably altered but if the antigen was injected following several daily exposures antibody formation was suppressed. In

subsequent studies using lethal levels of irradiation Hektoen (1918, 1922) clearly demonstrated suppression of lysin and precipitin production.

Although other early investigators (Konrich, 1925; Paulin, 1925 and Lusztig, 1929) failed to observe suppression of antibody formation or reported an actual increase in antibody titers following x-irradiation the foregoing early results on suppression of antibody formation have been repeatedly confirmed during the last decade.

From the beginning of the work on irradiation, the importance of the time of irradiation with respect to immunization has been widely recognized. Fredell et al. (1948) noted that 550 r* total body irradiation given 10 days after antigen administration had a slight or insignificant effect whereas radiation given with antigen, depressed and delayed hemolysin production. Taliaferro et al. (1952) observed that hemolysin formation was inhibited when antigen was injected 12 hours to 24 days following radiation exposure (400, 500, or 700 r). But when antigen was injected 4 days before or 6 hours after irradiation peak hemolysin titers were not reduced, but the rate of antibody for ation was decreased. Kohn (1951) reported that x-irradiation, given up to 7 days following antigen injection suppressed peak titers in rats, the attainment of the peak was delayed, the decline in titer after the peak was delayed and larger doses inhibited hemolysin production more than small doses. Craddock and Lawrence (1948) noted that 250 r x-irradiation depressed antibody formation in rabbits if antigen was injected 8 hours following exposure but had no effect when administered 4 days after beginning immunization. Burrows, Deupree and Moore (1950) noted that irradiation resulted in sharply

*r=Roentgen

reduced serum antibody titer but produced a transitory stimulus of fecal or urinary antibody, apparent when immunization was begun at one day, but not 3 days, after irradiation.

Hale and Stoner (1952a, 1952b, 1953) found that gamma radiation with Co-60 inhibited production of neutralizing antibody to influenza virus, tetanus antitoxin and pneumococcus antibody. Silverman and Chin (1955) noted a delay in antitoxin production to tetanus toxoid in irradiated mice. Jacobson et al. (1949) and Jacobson, Robson and Marks (1950) observed that rabbits given 700 r developed no demonstrable hemolysin titer or low titer, for example, titers of 1:80 or 1:40 occurred on the twenty-first and twenty-eighth days respectively after immunization whereas titers of 1:5120 were observed on the fourteenth day in normal (non-irradiated) immunized rabbits; these levels declined to 1:160 on the twenty-eighth day.

The extensive literature concerning the effects of x-irradiation on antibody production has also been reviewed by Taliaferro and Taliaferro (1951) and Hale and Stoner (1954).

A considerable amount of experimental data indicates that antibody response may be divided into an early radiosensitive phase and a late radioresistant phase. Dixon, Talmage and Maurer (1952) reported that 400 r whole body irradiation given at the same time or 6 hours after injection of radioactive iodinated bovine gamma globulin had little effect on antibody response. Similarly 800 r given 3 days after antigen failed to suppress the antibody response. Contrary to these findings they reported that 400 r given 5 hours before the antigen reduced antibody response, and immunization 12 to 48 hours after exposure completely inhibited it. On the basis of these results, the authors postulated

two phases in antibody synthesis. The first phase, the initiation of antibody synthesis, appeared to be completed with the first 6 hours after injection of the antigen since animals injected 6 or more hours after irradiation showed complete inhibition of antibody formation; the second phase, during which antibody was formed by the host, was believed to be radioresistant. Data which in part support such a hypothesis has been collected by Kohn (1951), Taliaferro et al. (1952), Smith and Gump (1953), Maurer et al. (1954), Taliaferro and Taliaferro (1954a and 1954b).

Additional information with regards to antibody formation and x-irradiation comes from the work of Craddock and Lawrence (1948), Taliaferro et al. (1952), Dougherty and White (1946), Dixon et al. (1952), Silverman and Chin (1954) and Crosland-Taylor (1955) on secondary antibody response. Investigations have been reported by Taliaferro and Taliaferro (1957) and Smith et al. (1958), on the effect of repeated or intermittent exposure to x-irradiation. The effect of shielding of various organs and regions of the body, and spleen and bone marrow transplants on antibody production have been reported by Jacobson et al. (1950), Jacobson and Robson (1952), Wissler et al. (1953), Taliaferro and Taliaferro (1956), Sussdorf and Draper (1956), LaVia et al. (1957), Makinodan et al. (1956), Gengozian and Makinodan (1956).

The available literature all supports the proposition that inhibition by x-irradiation of antibody formation depends on the irradiation dose and the time elapsing between administration of the antigen and exposure to x-irradiation. At those levels of irradiation where tissues responsible for antibody production are not irreversibly impaired, antibody production is delayed or inhibited. Both the time

interval between the administration of the antigen and exposure to irradiation during which injury occurred, and the time necessary for recovery of the antibody-forming mechanism are dependent upon the dose of irradiation. The higher the dose, the greater the amount of inhibition and the longer the period necessary for recovery.

As to alteration, destruction or functional impairment of preformed potentially protective antibodies by irradiation, the evidence available adequately indicates that this apparently is not a factor of importance at radiation levels below an LD_{50} for the various animals under observation. In early studies Hektoen (1915) noted that x-irradiation had no effect on antibody titers at "exposure levels which had no noticeable effects on the general condition of the rat." The Taliaferros (1950, 1952, 1954) reported that the declining trend of hemolysin titers in rabbits was not altered by exposure to radiation doses less than the LD_{50}. Craddock and Lawrence (1948) in a study of the anamnestic response of rabbits to typhoid H and O antigens and for sheep erythrocytes, found that a 250 r dose of x-irradiation did not alter antibody titers. Hollingsworth (1950) noted that "x-irradiation in dosage of 300 r had no effect on the rate of destruction of heterologous antisera given intravenously to rabbits 24 hours after irradiation". Additional evidence has been presented by Silverman and Chin (1955) in experiments designed to determine the relationship of the time of irradiation on the development of antitoxic immunity. They reported that mice immunized seven days prior to x-irradiation were able to resist a later challenge injection of tetanus toxin. Less direct evidence reported in other studies (Smith et al., 1954; Hatch, 1954 and Paullisen and Shechmeister, 1955) supported the contention that preformed antibody is not affected by irradiation.

B. Effect of Irradiation on Phagocytic Function

1. Effect of irradiation on the cells of the reticulo-endothelial system. Total body x-irradiation has been reported to decrease, have no effect on, or increase phagocytosis by reticuloendothelial cells as measured by blood clearance and organ distribution studies.

Chrom (1935) observed that following intravenous injection of bacteria into mice that the bacteria were cleared from the blood of irradiated animals more slowly than in normal animals. If the spleen was protected by lead shielding during x-irradiation the rate of disappearance of bacteria from the blood stream could be enhanced.

Gordon, Miller and Hahne (1953) and Gordon et al. (1955) reported that after intravenous injection of viable Klebsiella pneumoniae that bacteria disappeared from the blood almost as rapidly in irradiated animals as nonirradiated controls during the first 4 hours, but that after 8 hours the bacteria increased in the blood stream of the irradiated animals and resulted in their death.

Taplin et al. (1954) using blood retention of intravenously injected prodigiosin as an index of phagocytic function reported that exposure of rabbits to doses of 800-1200 r (LD_{50} - LD_{100}) whole body irradiation resulted in an increased blood retention of the dye, whereas 300 r did not alter retention of prodigiosin. Shielding of the liver and spleen area with lead while irradiating the rest of the body with 1200 r resulted in normal removal of the injected prodigiosin.

Di Luzio (1955) observed depression in the uptake of intravenously injected colloidal gold by rat liver following x-irradiation (1040 r - LD_{100}) when tested on the fourth postirradiation day.

Investigations which have revealed no difference in either disappearance (clearance) rates or organ distribution have been reported. Barrow et al. (1951) determined the 50% disappearance rate of intravenously injected radio-active colloidal gold from the blood stream of both normal and x-irradiated (800 r) rabbits. These workers found that the rate of phagocytosis was not significantly impaired between groups of normal and x-irradiated animals and the rate of blood clearance was no different in the same rabbit before or after irradiation exposure.

Esplin, Marcus, and Donaldson (1953) reported no significant difference in the 72 hour uptake of colloidal ThO_2 by the mouse spleen following total body exposure of 100-600 r. Similarly Gyi and Marcus (1957) have reported that exposure of mice to whole-body x-irradiation in doses of 300, 400, or 500 r did not affect phagocytosis of intravenously injected ThO_2 by reticulo-endothelial cells of the spleen when determined two days following irradiation. However, when measurements were carried out on the seventh postirradiation day similar results were obtained at the 300 or 400 r level of exposure but radiation in a dose of 500 r significantly depressed the 2, 6, and 24 hour uptake of ThO_2 by mouse spleen.

Fitch et al. (1953) studied the distribution of radioactivity in various organs of rats and also the rate of disappearance of I-131 labeled typhoid organisms from the blood stream. These investigators were unable to demonstrate any significant differences in either disappearance rates or organ distribution between normal and x-irradiated (500 r) rats.

Ingraham (1955) reported that x-irradiation (500-600 r) of rabbits failed to alter the rate or extent of removal of S-35 labeled sheep

erythrocytes stromata from the circulation when tested on the first and third postirradiation day. Furthermore, the retention of radioactivity by the liver, spleen, kidney and bone marrow was unaffected.

Gabrieli and Auskaps (1953) studied the effect of 25, 50 and 100 r whole body irradiation on the disappearance rate of intravenously injected radioactive colloidal chromium orthophosphate ($CrP-320_4$) in rats. These investigators reported no difference in blood clearance among irradiated and control animals.

At least one group of investigators (Wish et al., 1952, reported an increase in blood clearance rates following whole body x-irradiation. These workers injected I-131 labeled homologous and heterologous plasma P-32 labeled homologous and heterologous erythrocytes, colloidal gold containing Au-198 and Evans blue, into the circulation and reported that all these substances disappeared faster from the circulation of x-irradiated ("rabbits received 1000 r and mice 575 r of roentgen rays, an approximate LD_{50} dose") mice and rabbits, than from the blood streams of normal animals. The suggestion was made that irradiation injury resulted in increased capillary permeability which could account for the increased disappearance rates observed.

2. Effect of irradiation on phagocytic leukocytes. Hematological patterns following radiation has been present by Bloom (1948), Eldred and Eldred (1953), Jacobson et al. (1949) and Cronkite and Brecker (1955). The main phagocytic elements of peripheral blood (neutrophils and monocytes) dramatically decrease in number following x-irradiation. The magnitude and duration of these changes are directly related to the irradiation dose.

Sheckmeister, Paulissen and Yunker (1956) reported that a marked peritoneal leukocytosis occurred in both normal (nonirradiated) and irradiated (300 r) mice one day after irradiation following initiation of a peritoneal inflammatory response but for the irradiated animals this response lasted only 2 days, declining precipitiously on the third day to remain at a low level during the remaining 7 days of observation. Similarly, irradiated animals peripheral white blood counts dropped rapidly resulting in a marked leukopenia.

Speirs (1956) observed that irradiation (500 r) given prior to antigen injection greatly reduced the number of eosinophil and mononuclear cells in the peritoneal fluid and prevented an increase of these cells in response to specific antigen in both immunized and non-immunized mice.

Esplin, Marcus and Donaldson (1953) observed a marked reduction of leukocyte response in the peritoneal cavity of x-irradiated mice. These investigators noted that smears of peritoneal fluid obtained from x-irradiated mice consistently showed more free bacteria than those obtained from normal mice. Although they observed that the percentage of active peritoneal phagocytes were increased, total phagocytosis was less in the x-irradiated animal.

Knott and Watt (1929) studied the <u>in vitro</u> effect of x-irradiation on blood cells. They irradiated blood samples from normal and leukemic patients in a paraffin wax chamber and observed a decrease in phagocytic activity of leukocytes in both normal and leukemic blood within 35 minutes after irradiation. Similarly "within about 20 minutes the percentage of actively phagocytic polymorphs began to fall" in patients who received deep radiation therapy.

Ingram and Adams (1952) measured phagocytosis by the percentage of leukocytes containing iron granules after incubation of heparinized blood with saccharated iron. They found that irradiation (300 r) produced a decrease in phagocytic activity one week after exposure.

Fishman et al. (1953) studied the role played by leukocytes in the increased susceptibility of rats to infection. These investigators determined the activity of phagocytes from blood samples of normal and irradiated (600 r) animals at various postirradiation periods using Staphylococcus aureus as the test organism. A higher phagocytic index than normal was noted 2 and 12 hours postirradiation. This was followed by a below-normal phagocytic index beginning on the third and extending to the eleventh postirradiation day.

Shechmeister and Fishman (1955) observed that exposure of rats to 600 r total body irradiation did not influence either the rate or the extent of leukocyte migration one day after irradiating but did decrease migration on the second and the fifth postirradiation day. These authors (Fishman and Shechmeister, 1955) pointed out that effective phagocytosis is governed not only by the number of granulocytes and the extent of their migration but also by their ability to ingest and finally digest the engulfed bacteria. They observed increased phagocytic indices 2 and 12 hours after irradiation, accompanied by leucopenia, primarily lymphopenia. One day after irradiation the phagocytic activity returned to normal concomitant with normal migration of leukocytes. On the second postirradiation day the opsonophagic index and index of surface phagocytosis fluctuated between normal and subnormal values and leukocyte migration was slightly but significantly decreased. The gradual manifestation of radiation damage became more pronounced between the third

and fifth postirradiation days when white blood cell counts were lowest, opsonophagic and surface phagocytosis indexes were decreased and animals showed an increased susceptibility to infection.

Wilkinson (1954) noted that on the first, fifth and sixth post-irradiation days that the per cent of phagocytic neutrophils and the number of ingested bacteria per active phagocyte was higher in the blood of irradiated (550 r) rats. However, from the seventh until the thirteenth day he found a statistically significant decrease in the ability of leukocytes to carry on phagocytosis.

3. Effect of irradiation on intracellular digestion. Phagocytosis, to be effective, not only must consist of ingestion of the infective particles, but digestion, with ultimate destruction of the organism, must follow if protection is to be gained. The observations by Gordon et al. (1955) of re-entry of K. pneumoniae into the blood stream, 8 hours after initial injection suggested that although animals could effectively clear the circulation of the injected organisms, the ability of the phagocyte to destroy the ingested microbe could be impaired as a result of x-irradiation exposure.

Fitch et al. (1953) employed urinary excretion of the isotope following intravenous injection of I-131 labeled typhoid vaccine as a measure of degradation of the injection particles. Although the total isotope excretion was the same at the end of 6 days, the isotope excretion during the 24 hours following intravenous injection of the labeled organisms in x-irradiated animals was 47% of the radioactivity as compared to 68% I-131 excretion in normal animals.

Donaldson, Marcus and Gyi (1954) reported that x-irradiation (350 r) of mice resulted in decreased intracellular digestion of the

nuclei of chicken erythrocytes by peritoneal phagocytes during the second postirradiation week (sixth through fifteenth postirradiation day). When the irradiation dose was increased to 450 r, this depression of intracellular digestive capacity was extended through the third postirradiation week (twenty-second postirradiation day). These studies were extended (Donaldson et al., 1956) and it was observed that phagocytic digestion of Candida guilliermondi was inhibited following x-irradiation. This inhibition followed the same pattern as observed with chicken red blood cells. These authors noted that immunization resulted in an increase in the per cent of engulfed chicken erythrocytes undergoing digestion. However, this immunization-induced increase in digestive capacity was reversed by irradiation exposure.

C. Effect of Irradiation on Bactericidal Action of Serum

Marcus and Donaldson (1953a, 1953b) first demonstrated that the bactericidal activity of normal serum was decreased following irradiation exposure. These authors reported that following irradiation (650-700 r) rabbits developed, with rare exceptions, a measurable loss of serum bactericidal activity against B. subtilis near the end of the first postirradiation week and that this lowered serum bactericidal capacity had returned to normal by the twentieth postirradiation day. If rabbits were given a second 650 r dose of irradiation 28 days following initial exposure, the decreased serum bactericidal function developed sooner and remained depressed for longer periods of time. Subsequent studies (Donaldson and Marcus, 1953b) revealed that the addition of serum from irradiated animals to normal serum did not inhibit bactericidal activity of normal serum. These authors reported that natural

agglutinin and complement concentrations of the serum were unchanged by irradiation doses that resulted in suppression of serum bactericidal activity. Hematological studies carried out simultaneously with serum bactericidal studies (Donaldson, 1954) revealed that the depressed serum bactericidal activity occurred at times when circulating leukocytes and platelets were at low postirradiation values.

Fishman and Shechmeister (1955) reported that the bactericidal power of the blood of x-irradiated (600 r) rats was depressed for M. aureus when measured 3 and 6 days after exposure to x-rays and had returned to normal 30 days after irradiation.

Pillemer et al. (1954) isolated "properdin" a normal serum protein and suggested that it was a factor of importance in innate resistance since it possesses nonspecific bactericidal activity against B. subtilis and a variety of Gram negative species to properdin is variable, depending upon the age of the culture and the particular strain employed (Wardlaw and Pillemer, 1956).

Serum properdin levels decrease following x-irradiation (Ross et al., 1955; Ross, 1956; Linder, 1957). This fall in normal serum properdin levels in irradiated animals has been suggested as an important factor in the development of fatal postirradiation bacteremia (Ross, 1956).

In contrast to these findings early work by Fried (1926) reported an antibacterial agent in serum of irradiated patients and Koga (1933) observed an increase in bactericidal activity within an hour in irradiated dogs, which reached a maximum in 3 to 6 hours and returned to normal in about 24 hours.

D. Effects of Active Immunization on Resistance to Infection or Irradiated Animals.

Insight has been gained from the previous considerations of the effect of x-irradiation on the isolated systems which function as protecting agencies; however, the role immunization plays in augmenting resistance is yet to be determined in many instances for the x-irradiated animal. Here the reports in the literature are scanty and, although not in complete agreement, seems to indicate that active immunization may enhance the resistance of the irradiated animal.

Fulton and Mitchell (1953) observed that x-irradiation (200 r) 24 hours prior to challenge completely destroyed the resistance of mice to Salmonella typhimurium, and in effect, converted immune animals into non-immune animals. However, these investigators recognized the level of x-irradiation required to completely destroy resistance of mice to S. typhimurium to be dependent upon the degree of immunity conferred by the immunizing procedure. The vaccination procedure used by these investigators consisted of a single intraperitoneal injection of the antigen suspension; hence, a high level of protection might not be expected.

In contrast to these results Paulissen and Shechmeister (1955) demonstrated that x-irradiation caused an increase in resistance and a more rapid recovery of resistance in the challenged irradiated mice. Smith et al. (1954) found that immunization with Proteus organisms prior to LD_5 irradiation increased resistance of mice and rats challenged with the living organism. Immunization with mixtures of bacteria which occur most frequently in post mortem blood also increased resistance to challenge. However, immunization with these mixtures did not increase

the survival of animals when the irradiation dose was increased to an LD_{65-95}.

Immunization against P. morgani offered protection against infection resulting from challenge three days after x-irradiation with 525 r. Hatch (1954) observed mortality results of 7% for both the x-ray and challenge control groups, 100% for the irradiated-challenged group and only 55% for the immunized challenge group.

MATERIALS AND METHODS

I. Animals

Standard laboratory animals of both sexes, the mouse, rat, and rabbit have been employed as test animals in the course of this investigation. Adult albino mice (Mus musculus) were obtained from a regional source and weighed between 20-30 gms. Young adult albino rabbits weighing 3-4 kgs and adult albino rats (Sprague-Dawley) weighing 200-300 gms were procured from reputable local sources.

II. X-irradiation

A Westinghouse Quadrocondex x-ray machine was used as the source of irradiation in all experiments. Physical factors were; 250 KV, 15 ma, 1.0 mm Al and 0.5 mm Cu filters in addition to an inherent filtration of 2.5 mm Al and 0.25 mm Cu. The distance from focal point to surface on which experimental animals were placed was 50 cm for rats and mice and 105 cm for rabbits. Mice were irradiated in groups of twelve or less in a cylindrical container having a diameter of 17 cm and a height of 3 cm. Rats were irradiated 3 or 4 at a time in a similar container having a diameter of 23 cm and a height of 5.5 cm. Rabbits were irradiated in pairs, in a wooden box with dimensions of 8 x 16 x 7.5 inches. All animals received total body exposure. The average dose rate as measured by a Victoreen r* meter in air at the same location as the

*r= Roentgen - is that quantity of photon radiation, when incident on a cubic centimeter of dry air at standard conditions produces secondary electron radiation which, if completely absorbed in air will give rise to a total charge of one electrostatic unit (1 esu).

center (horizontal) of the experimental animal's body was 90 r per minute for rats and mice. An average dose rate of 29.6 r per minute was determined for rabbits by measuring irradiation dose in different parts of a wax phantom which occupied the same position as the rabbit.

III. Determination of Serum Bactericidal Activity

The usual procedure for testing serum bactericidal activity was to make a 1:10 dilution of sterile serum using physiological sodium chloride (saline) as the diluent. The total volume in all tubes was adjusted to 1.8 ml. Two-tenths milliliter of a suitable dilution of a broth culture of the test organism was then added. The tubes were shaken and then incubated in a 37°C water bath for 1.5 hour. Pour plates were then prepared using 1 ml aliquots of appropriate dilutions of the incubated serum-organism suspension. Plates prepared in this manner were then incubated at 37°C for 24-36 hours and colony counts made.

IV. Testing for Bacteremia

The presence or absence of bacteremia was determined by streaking half a milliliter of whole blood (obtained aseptically by cardiac puncture) across the surface of a blood agar plate or by the addition of this same volume of blood to 5 ml tubes of tryptose phosphate (Difco, Detroit, Mich.) broth. The procedure was carried out in duplicate. One plate or tube was incubated aerobically and the companion plate or tube was incubated anaerobically. With mice the quantity of blood inoculated was reduced to 0.2 ml instead of the customary 0.5 ml for rats and rabbits.

V. Preparation of Vaccines

The vaccines used in all of the experiments to be described were prepared in the following manner:

K. pneumoniae vaccine: Vaccines were prepared from broth cultures. A stock culture, kept in the refrigerator, was streaked to blood agar plates. Smooth mucoid colonies were picked after 24 hours incubation and passed two more times by the same procedure. Tryptose phosphate (Difco) broth was inoculated from the blood agar plates and, after 16 to 18 hours at 37°C formalin was added to yield a final concentration of 0.5%. The organisms remained in contact with the formalin for 24 hours and were then washed twice (saline) by centrifugation. The sediment was resuspended to yield a heavy suspension about equal in turbidity to McFarland scale number 10. Viability tests (culture on agar and in broth) with vaccines so prepared were uniformly negative.

S. typhimurium vaccines. Vaccines were prepared by harvesting 12-14 hour tryptose phosphate (Difco) broth cultures of *S. typhimurium* by centrifugation. Precipitates were washed 3 times (saline), concentrated and resuspended in saline (McFarland no. 10). Organisms were then killed by heating in a 56°C water bath for 2 hours or until viability tests proved negative.

E. coli vaccine. Vaccines were prepared after the method of Edwards (1951). Organisms were grown on tryptose phosphate (Difco) agar plates and removed by washing with saline. This suspension was heated at 100°C for 2 hours, sedimented with centrifugation, suspended in absolute alcohol and incubated for 24 hours at 37°C. Bacteria were sedimented again, resuspended in absolute alcohol and incubated for an

additional 24 hours at 37°C. These organisms were then centrifuged, washed 2 times with acetone and dried at 37°C. The dried cells were then weighed and resuspended in sterile saline for injection. Two strains of E. coli (one hemolytic and one non-hemolytic) isolated from lethally irradiated mice by cardiac puncture antimortem were subcultured for preparation of this antigen.

VI. Harvesting Cellular Exudates

Peritoneal exudates were induced in mice by injection of 1.0 ml of a glycogen-saline solution (0.1 mg/ml). Forty-eight hours after, at a time when the majority of the cells in the peritoneal cavity were macrophages, these cells were harvested by injection of 4-5 ml of heparinized phosphate-buffered saline and aspiration of the exudate. Peritoneal exudates were induced in rabbits by injection of 500 ml of 1.0 mg/ml glycogen-saline solution. Forty-eight hours later rabbits received a second 500 ml infusion of heparinized phosphate-buffered saline. One hour later exudates were harvested by aspiration.

The cells of the peritoneal exudates were washed three times with phosphate buffered saline (pH 7.2) and resuspended in this medium in appropriate concentrations for injection into x-irradiated mice or suspended in Krebs-Ringer phosphate rabbit serum buffer (one part normal rabbit serum to two parts Krebs-Ringer phosphate buffer, pH 7.4) for use in Warburg (manometric) studies. All cell handling procedures were carried out in the cold to repress growth from possible contaminating organisms.

VII. Manometric Technique

Warburg's direct method of measuring oxygen-uptake as outlined by Umbreit, Burris and Stauffer (1956) was employed in these studies. Tests were conducted in 20 ml Warburg flasks. The main flask compartment contained a 2.9 ml aliquot of an appropriate dilution of phagocytic cells suspended in Krebs-Ringer phosphate rabbit serum buffer. The center well of each flask contained 0.2 ml of 10% potassium hydroxide to absorb the liberated carbon dioxide. Small pieces (one square cm) of accordian folded filter paper were placed in the center wells. The gas phase was air. The final step in this procedure was the addition of 0.1 ml of the test organism. The working hypothesis was enunciated that changes in oxygen uptake could be correlated with death or proliferation of ingested microorganisms in phagocytic cells. The incubation temperature was 37°C ± 0.5°C. The oscillation rate was 100 cycles per minute with an amplitude of 2.5 cm. After an equilibration period of 15 minutes the system was closed and oxygen-uptake readings were obtained at given time intervals.

VIII. Challenge of Experimental Animals

Oral Challenge. Challenge was carried out by instilling known numbers of organisms into the stomach by means of a catheter adapted from a 21 gauge hypodermic needle which was attached to a tuberculin syringe.

Aerosol challenge. The chamber utilized for challenge was a modification of the model described by Coburn et al. (1954) and is shown in Figure 1. Suspensions of organisms were introduced into the chamber by

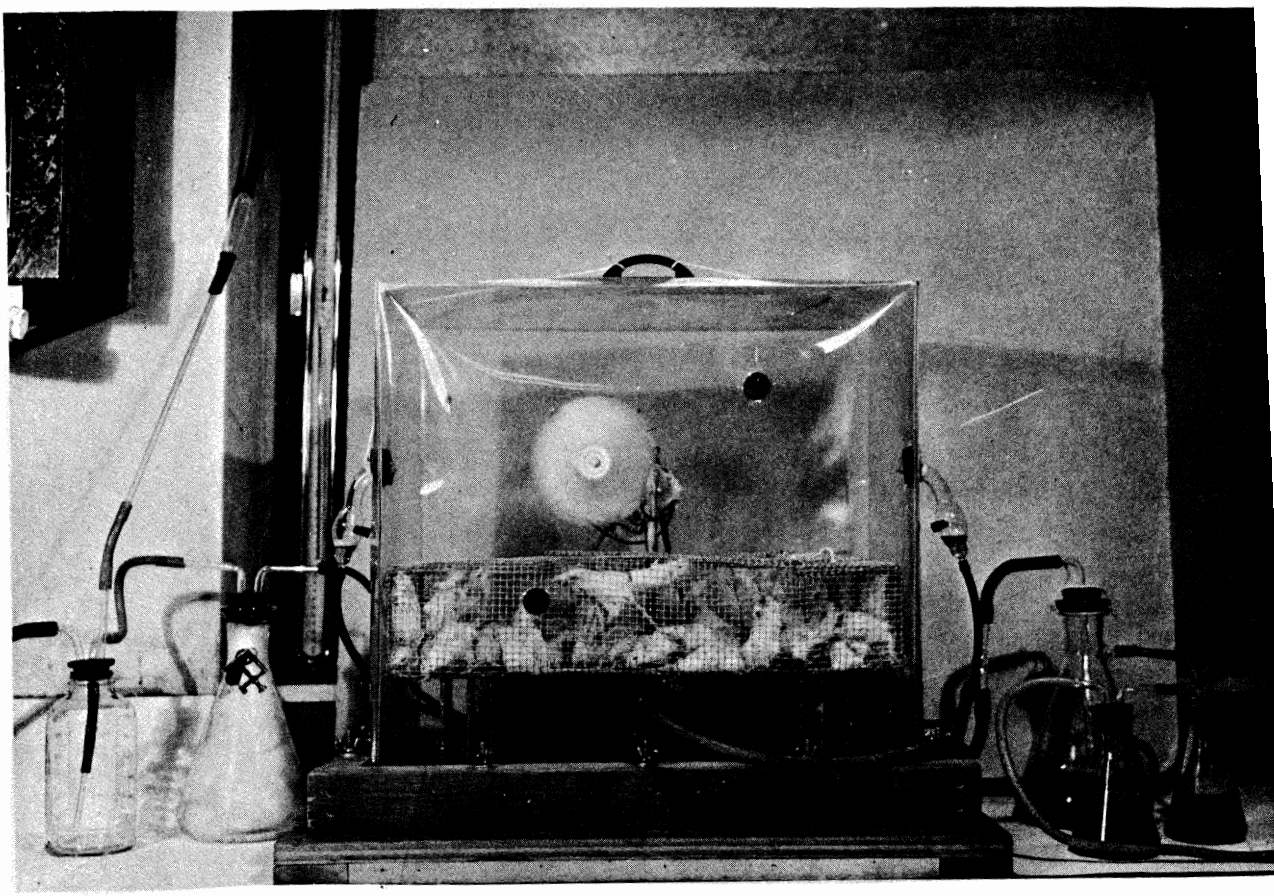

Figure 1

Aerosol chamber. See text for description of operation.

two DeVilbiss no. 40 nebulizers operated by a stream of compressed air at a positive pressure of approximately 60 mm Hg. Within the chamber a small fan ran continually during spraying. Tryptose phosphate (Difco) broth containing 1.5 ml of a suspension of approximately 2.3 x 10^8 infecting organisms per ml, as estimated by plate count, was aerosolized in each nebulizer during a 30 minute period. Mice were contained in a single wire compartment 4 inches high and fitting the inside dimensions of the chamber supported at a level midway between inlet and outlet ports. In each experiment, animals from all experimental groups were exposed simultaneously.

Parenteral challenge. Challenge was carried out by the conventional intraperitoneal, subcutaneous and intravenous routes by means of a 26 gauge meedle attached to a tuberculin syringe.

Intratracheal instillation. The method employed for intratracheal instillation was that described by Jourdonais and Nungester (1935) with modifications suggested by Gunn (1955). The ether anesthetized rat was placed in a supine position with the head of the animal at the edge of the specially constructed operating block designed to elevate the head (Figure 2). The upper incisor teeth of the animal were hooked over a wire yoke which held the rat in the desired position. The tongue was then grapsed with the fingers and traction applied so that it was held firmly to one side of the mouth and against the lower jaw. The blades of a curved hemostat were then inserted far back in the pharynx and the root of the tongue raised by the spreading of this instrument. The field was illuminated by light from a head lamp. Under direct vision, a cannula was then inserted into the trachea. The cannula was constructed of brass tubing, approximately 7 cm long, 2 mm

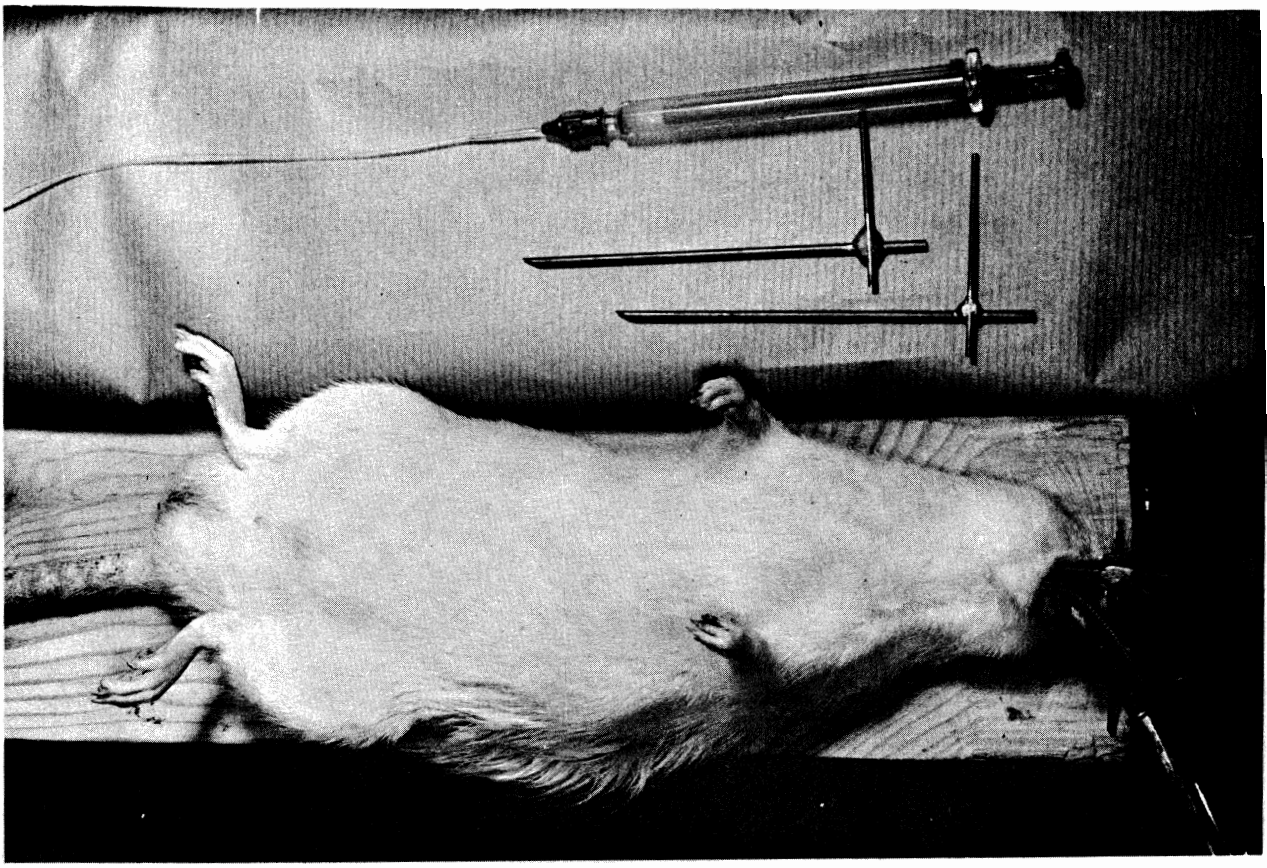

Figure 2

Tracheal intubation of the rat.

outside diameter, and 1.5 mm inside diameter. The tip of the tubing was beveled on the lateral surface. Near the proximal end of this tube a brass rod was soldered at right angles to the tube and served as a handle. To enter the trachea, the beveled tip of the cannula was placed just under the epiglottis; the proximal end of the instrument was depressed and, as the epiglottis opened with respiration, the cannula was inserted into the trachea. When the cannula was in the trachea, a drop of 0.5% mucin was placed over the exposed end to determine if the rat was breathing through the cannula. If the cannula entered the esophagus, small bubbles were formed but did not break on expiration. Once in the trachea the cannula was turned so that the beveled edge faced the right lung and gently passed into the left main stem bronchus. A short segment of polyethylene tubing was then passed through the lumen of the cannula and material instilled by means of a tuberculin syringe.

IV. Hemagglutination Technique

Blood was collected from experimental animals and serum separated immediately. All serums were heated in a 56°C water bath for 30 minutes; dilutions of 1:20 to 1:5120 were prepared with saline. The antigen used was washed human type A erythrocytes, 0.2% in saline. Antigen suspensions (0.5 ml) was then added to an equal volume of the serum dilutions. The mixtures were incubated in a 37°C water bath for sixty minutes, stored at 4°C overnight and hemagglutinin titers read.

X. Other Materials and Methods

The foregoing procedures and materials are to be considered standard unless modification of these methods are specified in the discussion of the experimental results. For purposes of continuity certain other materials and methods employed in the experiments to be reported have been placed in the experimental results.

EXPERIMENTAL RESULTS

I. THE ENHANCEMENT OF RESISTANCE OF X-IRRADIATED ANIMALS BY PREIRRADIATION IMMUNIZATION

Mary investigators have demonstrated that radiation-induced injury enhances susceptibility to infection. With moderate exposure (i.e., in the LD_{50} range) infection plays a dominant role and is usually regarded as an important cause of death in the radiation syndrome.

It seems apparent, therefore, that active immunization prior to x-irradiation might afford protection to these animals in which death is attributable to overwhelming infection. The rationale of such an approach might be questioned since other specific host defense mechanisms have been shown to be impaired following x-irradiation (Shechmeister and Fishman, 1955; Donaldson et al., 1954; Marcus and Donaldson, 1953, and Fishman and Shechmeister, 1954. However, Smith et al. (1954), Hatch (1954), and Paulissen and Shechmeister (1955) have reported protection following immunization against infection experimentally induced via subcutaneous and intravenous routes in x-irradiated mice.

The following experiments were undertaken to determine whether immunization prior to x-irradiation would significantly increase resistance of irradiated animals challenged by methods simulating natural routes of infection.

A. Intratracheal Instillation of Infectious Material into Normal and X-irradiated Rats Following Immunization

The purpose of this experiment was to determine if preradiation immunization would enhance the ability of the rats to resist a post-radiation challenge of K. pneumoniae instilled directly into the lung. Animals were immunized by giving five 0.5 ml intraperitoneal injections of a formalin-killed neopeptone glucose broth culture containing approximately 3.6×10^8 K. pneumoniae organisms per milliliter at two-day intervals with one week elapsing between the final injection and radiation exposure. These animals were divided into 2 groups and paired with 2 non-imunized groups. One of the paired (immunized-non-immunized) groups was then exposed to 450 r wholebody x-radiation 6 days prior to intratracheal challenge; a second paired group received no x-radiation and served as "challenge controls"; and a third unpaired group received x-radiation but no other treatment and served as "x-irradiated controls."

The 30-day mortality results following challenge with K. pneumoniae are presented in Table I. Immunization failed to enhance the resistance of either normal (nonirradiated) rats (groups 2 and 2a) or x-irradiated animals (groups 1 and 1a) to infection. X-radiation alone yielded an LD_{20} (group 3) for these animals and markedly increased their susceptibility to infection with the challenge organism (compare groups 1a and 2a).

Since the immunizing procedure with this vaccine failed to confer even a slight degree of immunity upon the rat, a vaccine (K. pneumoniae vaccine prepared as described in Materials and Methods, Section V) was prepared for use in the following experiment. The immunizing procedure again consisted of giving five 0.5 ml intraperitoneal

TABLE I

EFFECT OF IMMUNIZATION ON SURVIVAL OF NORMAL AND X-IRRADIATED (LD_{20}) RATS EXPERIMENTALLY INFECTED* WITH K. PNEUMONIAE

Group	Number of rats	Preradiation treatment	X-radiation	Mortality (percent)
1	20	Immunization	450 r	90
1a	20	None	450 r	100
2	20	Immunization	None	55
2a	20	None	None	45
3	20	None	450 r	20

*Challenge inoculum - 0.1 ml of 2.5 percent gastric mucin suspension containing 476 ± 8 K. pneumoniae organisms; challenged on sixth post-radiation day.

injections at 2-day intervals, with one week elapsing between the final injection and x-radiation exposure. In addition, the experimental design was altered so that the dose of x-radiation delivered would be just sublethal and the K. pneumoniae challenge alone would not exceed an LD_{25} in normal (nonirradiated) animals. By keeping these factors at a minimal level, it was assumed that if resistance developed but was of small magnitude, it would not be masked by a high mortality resulting from either or a combination of these potentially lethal forces.

The mortality results following this immunization x-radiation challenge procedure are recorded in Table II. It can be seen that again immunization of normal (nonirradiated) rats failed to afford protection against the K. pneumoniae challenge (compare groups 22 and 22a). As could be predicted, this immunization procedure was not of protective value in x-irradiated animals (Table II, compare group 11 and 11a).

Agglutinin titers with the sera of immunized and normal rats are presented in Table III. A relatively small but definite increase in agglutinin titers was noted among the immunized rats.

The data presented neither support nor reject the hypothesis that preradiation immunization with K. pneumoniae enhances the resistance of x-radiated rats to subsequent intratracheal challenge with living organisms since the immunizing procedures employed failed to afford protection to normal animals.

An important ancillary finding in the course of this investigation was that preliminary experiments performed to determine the influence of intratracheal instillation of sterile suspending media on x-irradiated rats demonstrated that intratracheal inoculation of

TABLE II

EFFECT OF IMMUNIZATION ON

SURVIVAL OF NORMAL AND SUBLETHALLY

X-RADIATED RATS CHALLENGED* WITH *K. PNEUMONIAE*

Group	Number of rats	Preradiation treatment	X-radiation	Mortality (percent)
11	16	Immunization	400 r	37.5
11a	18	None	400 r	40
22	20	Immunization	None	20
22a	17	None	None	27
33	20	None	400 r	0

*Challenge inoculum - 0.1 ml of 2.5 percent gastric mucin suspension containing 392 ± 6 *K. pneumoniae* organisms; challenged on sixth post-radiation day.

TABLE III

ANTI-*K. PNEUMONIAE* AGGLUTININ TITERS

FROM SERA OF IMMUNIZED* AND NORMAL RATS

Group	Treatment	Number of rats	Number of rats giving antibody titers: 1:2	1:2	1:4	1:10	1:20	1:40	1:80	1:160	1:320
I	None	15	6	7	2	-	-	-	-	-	-
II	Immunized	15	-	-	-	-	-	6	6	1	2

*Five intraperitoneal injections (0.5 ml) every other day; sera were collected 14 days after final injection.

microorganisms can be utilized as a means of challenging animals via the respiratory route with assurance that if the dose of x-radiation is kept below an LD_{50} value, the effects obtained cannot be attributed to any detrimental effect of operative manipulation or the suspending media on x-irradiated rats. Therefore, mortality differences between control and irradiated animals challenged intratracheally must be attributed to invasion by the challenge organisms. However, if irradiation levels are of such magnitude that radiation alone results in mortality greater than LD_{50}, the method of intratracheal instillation is not feasible. Under these circumstances, introduction of sterile material into the lungs of x-irradiated animals results in infection by the endogenous flora (Figures 3, 4 and 5).

Since the immunological failure was attributed to the reaction of the species under investigation, the rat, this experimental animal was abandoned for this phase of the work and mice were then employed in succeeding challenge experiments.

B. Effect of Preradiation Immunization on Resistance to Aerosol-induced Infection in X-irradiated Mice

As the intratracheal instillation technic was not feasible in the laboratory mouse, the aerosol technic as described in the material and methods section of this thesis was adopted.

1. Effect of immunization on resistance to induced infection in irradiated mice. Adult albino mice were immunized by giving 5 intraperitoneal injections of K. pneumoniae vaccine at two-day intervals, with one week elapsing between the final injection and radiation exposure.

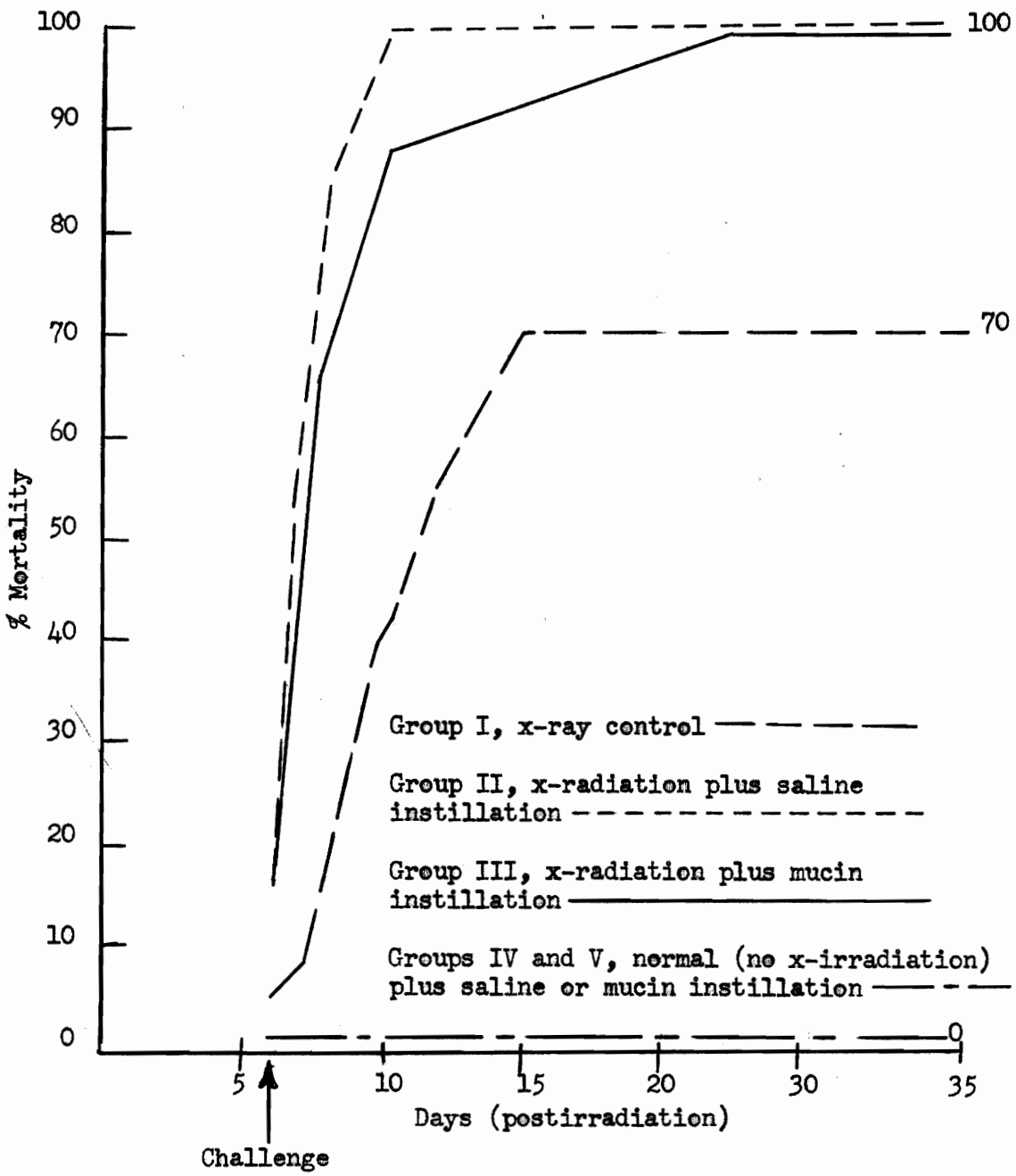

Figure 3

Cumulative mortalities of normal and x-irradiated (500 r) rats following intratracheal instillation of noninfectious material.

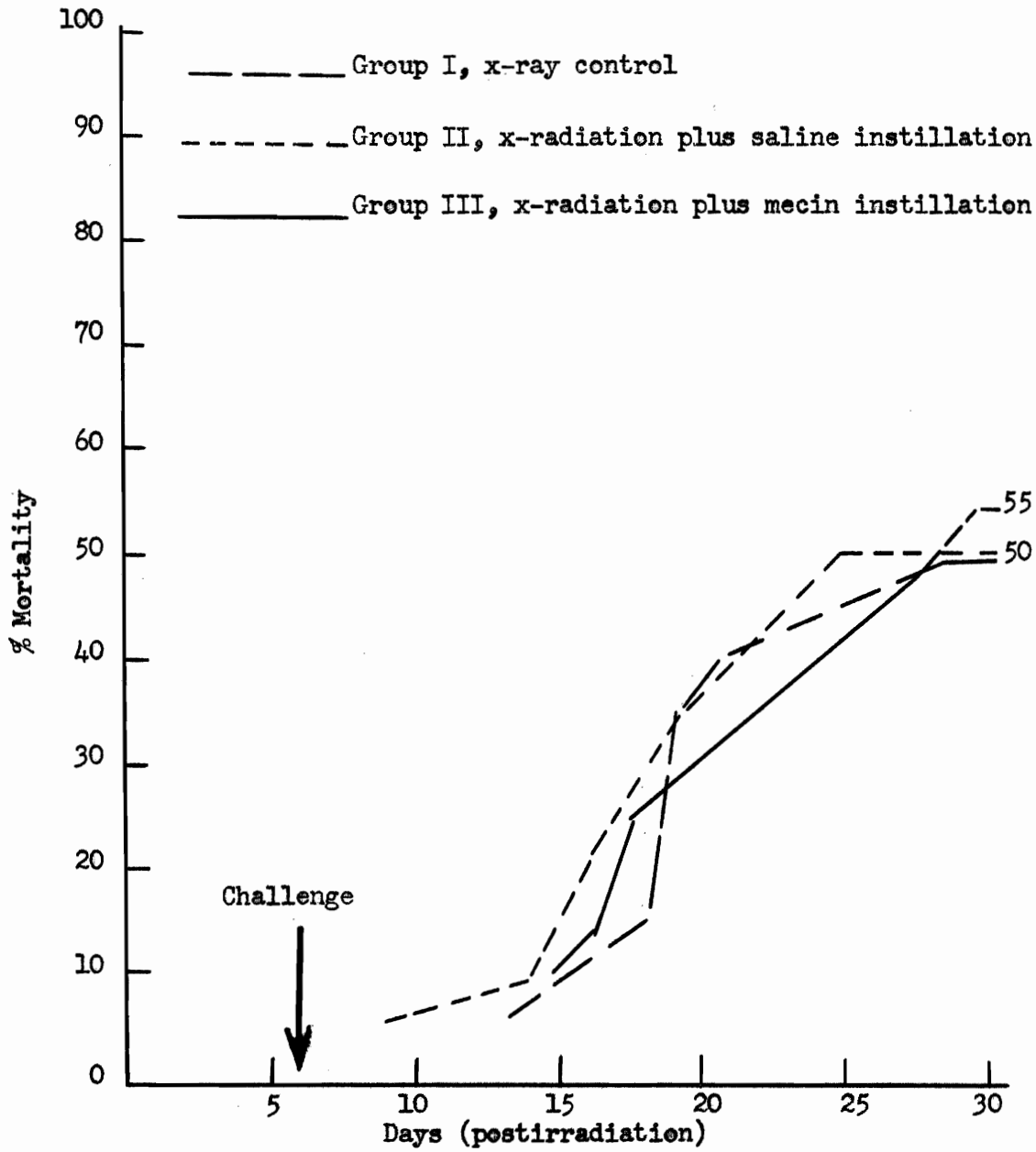

Figure 4

Cumulative mortalities of normal and x-irradiated (450 r) rats following intratracheal instillation of noninfectious material.

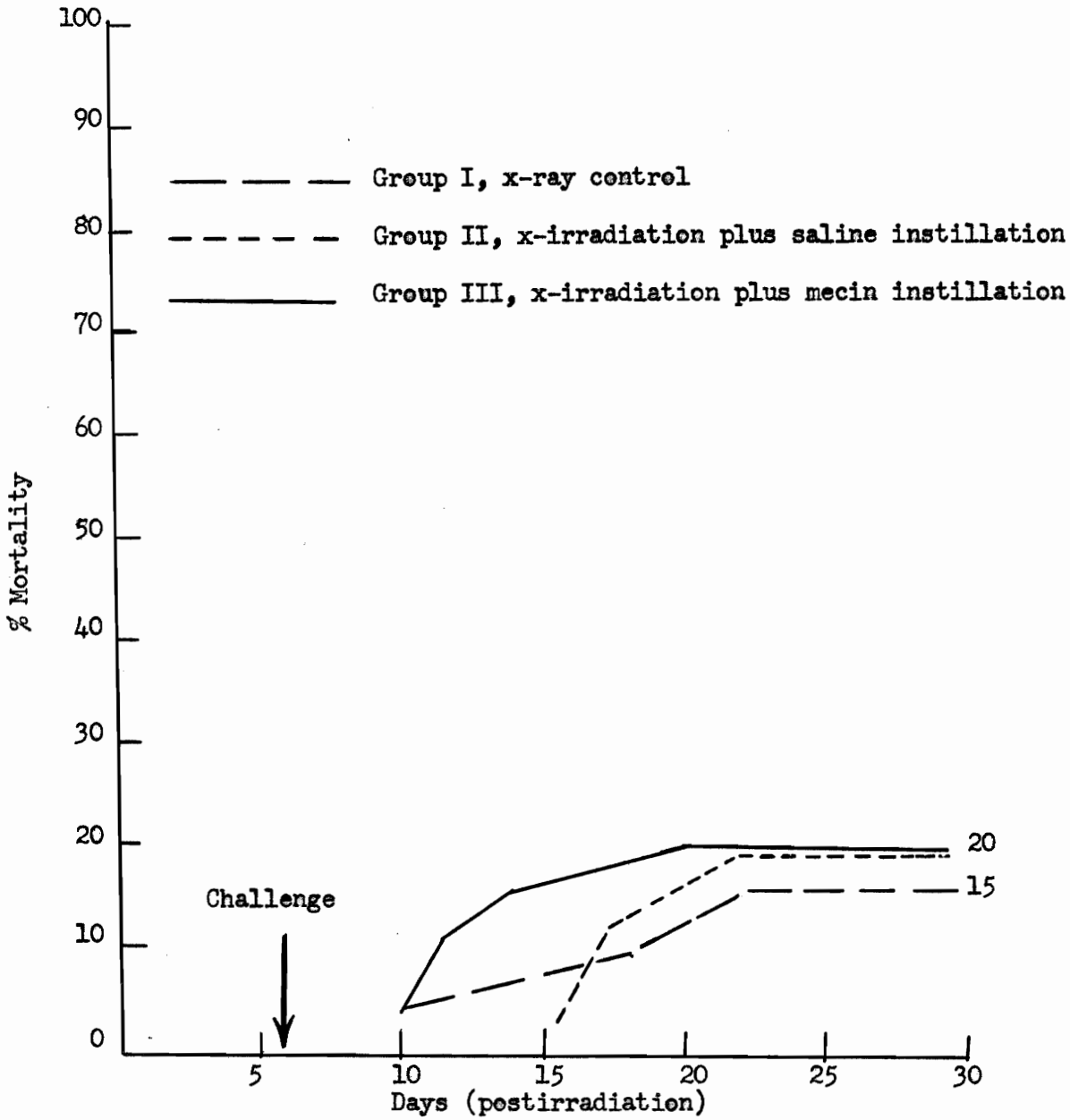

Figure 5

Cumulative mortalities of normal and x-irradiated (400 r) rats following intratracheal instillation of noninfectious material.

The animals were divided into 3 groups of 25 which were paired with 3 nonimmunized groups. One of the paired (immunized and nonimmunized) groups was then exposed to a 300 r total body x-irradiation dose 4 days prior to challenge. The second paired group was exposed to this x-irradiation dose and challenged 6 days later. The remaining paired group received no x-irradiation, and served as "challenge controls". Still another (unpaired) group received x-irradiation, but no other treatment, and served as the "x-irradiated control". Mice from all groups were then marked, and half of the animals from each group were placed in the chamber and challenged simultaneously. Immediately upon completion of the aerosol challenge, the remaining animals in each group were placed in the chamber and the procedure repeated. This permitted the animals of each group to be exposed simultaneously with organisms from the same broth culture. These organisms were nebulized at the same positive pressure, and all mice remained in contact with the aerosol spray for the same length of time. Following challenge, the mice were regrouped, caged, and daily mortalities recorded.

The 30-day mortality results following this immunization and x-irradiation challenge procedure are recorded in Table IV. All immunized groups have lowered mortalities when compared with paired nonimmunized control groups. Therefore, immunization prior to x-irradiation increased resistance and partially reversed the irradiation-induced increased susceptibility to the K. pneumoniae infection at the postradiation times employed.

Since mice which were immunized prior to LD_8 (300 r) irradiation were resistant to challenge, an effort was made to determine the x-irradiation exposures required to destroy such induced resistance to K. pneumoniae.

TABLE IV

EFFECTS OF IMMUNIZATION ON 30-DAY MORTALITY OF X-IRRADIATED (300 r) AND NORMAL EXPERIMENTALLY INFECTED WITH K. PNEUMONIAE

Group no.	Treatment	Days after x-ray mice were challenged	Percent Mortality*	P+
1	Immunized	6	36	0.2-0.1
1a	Normal	6	60	
2	Immunized	4	16	.001
2a	Normal	4	72	
3	Immunized challenge control	No x-ray	4	0.1-.05
3a	Normal challenge control	No x-ray	28	
4	X-ray control	No challenge	8	

*Each group contained 25 animals.
+Significant P values are underlined.

2. The effects of various levels of x-irradiation exposure on pre-induced resistance to K. pneumoniae. Adult albino mice were immunized as described before. One week after the last immunizing injection, these animals and nonimmunized control groups were exposed to either 200, 300, 400, 450, 500 or 600 r of total body x-irradiation. Four-day postradiation, experimental animals, with the exception of "x-ray control groups," were infected with K. pneumoniae by aerosol exposure.

The results obtained in this experiment are shown in Table V. Animals exposed to 200 r total body x-irradiation responded like normal nonirradiated animals (compare with immunized challenge controls which received no x-irradiation). Maximum difference between immunized irradiated mice and nonimmunized irradiated mice was observed at 300 r. At 400 r the degree of protection resulting from active immunization failed to be statistically significant at the 5 percent level (chi square technique) and at higher dose levels, the degree of protection was less significant but a trend toward protection was indicated. With animals exposed to 500 r of total body x-irradiation (LD_{88}) immunization afforded no protection against the induced infection.

3. The effects of chronic exposure on resistance. This portion of the study was undertaken to determine if cumulative damage of the host defense mechanisms resulted when mice were exposed to repeated low doses of x-irradiation over a comparatively long period.

Immunized and normal (nonimmunized) mice were exposed to 25 r of total body x-irradiation on alternate days 3 times weekly, over a 3 month period, until a cumulative dose of 800 r had been delivered. Twenty-four hours after the last exposure these animals were challenged with an aerosol of K. pneumoniae as previously described. It should

TABLE V

MORTALITY AT 30 DAYS OF IMMUNE AND NONIMMUNE MICE EXPOSED TO VARIOUS LEVELS OF X-IRRADIATION AND SUBSEQUENTLY INFECTED WITH K. PNEUMONIAE

Treatment	Radiation dose r	Mortality* %	P+
Immunization + x-ray + challenge	200	4	0.2-0.1
X-ray + challenge	200	24	
X-ray alone	200	0	
Immunization + x-ray + challenge	300	16	.01-.001
X-ray + challenge	300	56	
X-ray alone	300	8	
Immunization + x-ray + challenge	400	52	0.3-0.2
X-ray + challenge	400	72	
X-ray alone	400	32	
Immunization + x-ray + challenge	450	72	
X-ray + challenge	450	84	
X-ray alone	450	48	
Immunization + x-ray + challenge	500	96	
X-ray + challenge	500	100	
X-ray alone	500	88	
Immunization + x-ray + challenge	600	100	
X-ray+ challenge	600	100	
X-ray alone	600	100	
Immunized challenge control		4	0.1-.05
Normal challenge control		32	

*Each group contained 25 animals.
+Significant P values are underlined.
Aerosol challenge 4 days postradiation.

be noted that during the course of x-ray exposure only an occasional animal died; the total number of deaths over the 3 month period did not exceed the mortality in normal (non-irradiated) control animals.

Table VI summarized the treatment administered and records the 30 day mortality results following aerosol challenge. Immunized mice showed increased resistance to infection despite chronic radiation exposure. This observation confirmed the results of the initial experiments and demonstrated that active immunization afforded protection to mice exposed to ionizing radiation and subsequently challenged with the homologous organisms.

It will be noted that the nonimmunized, nonchallenged animals which received a cumulative dose of 800 r responded essentially in the same manner as mice exposed to a single 300 r dose of irradiation (Table V). Mortality results from these x-irradiation doses alone were similar (LD_8 acute and LD_{10} chronic). Also no difference in mortality existed between the groups of normal (nonimmunized) but aerosol challenged mice which received the different types of irradiation treatment (LD_{56} acute and LD_{60} chronic). Acutely irradiated (300 r) immunized animals, however, showed a greater resistance to bacterial challenge than chronically irradiated (800 r) animals (LD_{16} acute and LD_{33} chronic), although both had comparable antibody titers (Table VII).

These results clearly demonstrated that immunization with K. pneumoniae prior to x-irradiation increased the resistance of mice challenged with an aerosol of the living organisms. In an effort to augment and extend these observations, in order that generalizations might be made, challenge experiments using the oral route were conducted.

TABLE VI

MORTALITY AT 30 DAYS OF IMMUNE AND NONIMMUNE MICE SUBJECTED TO CHRONIC X-IRRADIATION EXPOSURE* AND SUBSEQUENTLY CHALLENGED WITH K. PNEUMONIAE

Group no.	No. of animals per group	Treatment	Mortality %	P+
1	33	Immunization + chronic exposure + challenge	33	0.1-.05
2	30	Chronic exposure + challenge	60	
3	20	Challenge alone (challenge control)	25	
4	20	Chronic exposure alone (x-ray control)	10	

*Dose of 25 r 3 times weekly; cumulative dose, 800 r.
+Significant P values are underlined.

TABLE VII

ANTI-*K. PNEUMONIA* AGGLUTININ TITERS OF SERA FROM IMMUNIZED X-IRRADIATED MICE

No. of serum samples*	Radiation	No. of serum samples with antibody titers of: 1:128	1:256	1:512
14	Acute exposure (300 r)	3	9	2
11	Chronic exposure (Dose of 25 r 3 times weekly; accumulative dose 800 r)	2	7	2

*Pooled samples of sera from 2 mice.

C. Effect of X-irradiation on Susceptibility to Infection Following Oral Challenge in Immunized and Nonimmunized Mice

Investigations by Miller et al. (1950, 1951) have demonstrated conclusively that invasion of the blood stream following exposure to x-irradiation is caused by organisms of the normal flora of the lower intestinal tract. The susceptibility of mice to infection by the oral route following ionizing radiation has been emphasized in recent investigations and the pathogenesis suggested (Perkins, Donaldson, and Marcus, 1956 and Gordon et al., 1955). In the present experiments the effect of immunization, prior to x-irradiation, on the ability of mice to withstand an orally administered challenge of Salmonella typhimurium or Bacterium tularense is reported.

1. Preliminary studies utilizing S. typhimurium. Young albino mice weighing 12 or 16 grams received three 0.1 ml intraperitoneal injections of vaccine (S. typhimurium vaccine prepared as described in Material and Methods, Section V) at 2 day intervals with 10 days elapsing between final injection and irradiation exposure. Animals were challenged 3 or 5 days postradiation with 0.5 ml of a 12 to 14 hr. broth culture of S. typhimurium containing approximately 3.5×10^8 organisms.

The results shown in Table VIII are expressed as percent mortality 3 weeks after challenge with S. typhimurium. Immunization prior to 350 r total body x-irradiation resulted in increased resistance among animals which were challenged either 3 or 5 days postradiation. Although this LD_{44} dose of irradiation greatly increased the susceptibility to infection of both immunized and normal animals, a statistically significant difference exists between these groups. Irradiation alone (group 4) resulted in 44% mortality and oral challenge of nonimmunized,

TABLE VIII

EFFECT OF IMMUNIZATION AND X-IRRADIATION (350 r)

ON SUSCEPTIBILITY TO INFECTION FOLLOWING ORAL

CHALLENGE WITH S. TYPHIMURIUM

Group	Preradiation treatment	Days after x-ray mice were challenged	Mortality %	P+ values
1a	Immunization	5	76	.05-.02
1b	None	5	100	
2a	Immunization	3	76	.05-.02
2b	None	3	100	
3	Challenge control	(No x-ray)	32	
4	X-ray control	(No challenge)	44	

*Each group contained 25 animals.
+Probability as calculated by Chi square (X^2) technique. Significant values at the 5% level are underlined.

nonirradiated mice (group 3) yielded 32% deaths. However, all irradiated and S. typhimurium challenged nonimmunized mice (groups 1b and 2b) died. This 100% lethal result exceeds the expected 76% mortality by a margin suggesting a true synergistic rather than additive effect of the two types of insult.

2. Effect of acute and chronic x-irradiation on induced resistance to S. typhimurium. Adult albino mice were given three 0.1 ml intraperitoneal injections of S. typhimurium vaccine at 4 day intervals, and 1 week later they were infected intraperitoneally with living organisms (approximately 1 LD_{50}). Thirty days later the survivors, which were considered immune, were paired with nonimmunized (control) animals and subjected to repeated small doses of x-irradiation. The animals were exposed to 25 r 3 times weekly for 4 weeks and thereafter to 33 1/3 r 3 times weekly for 6 weeks. The cumulative dose was 900 r for the 10 week period.

Meanwhile other mice were immunized with five 0.1 ml intraperitoneal injections of S. typhimurium vaccine on alternate days. One week after the last injection these animals and nonimmunized control animals were exposed to a single total body dose of either 300, 350, 400, 450, or 500 r.

Four days postradiation all experimental animals (acute or chronic exposure) with the exception of "x-ray control groups" were challenged by gastric instillation of 0.5 ml of a 12 to 14 hr broth culture of S. typhimurium containing approximately 517 x 10^8 organisms.

The 30 day mortality results of these experiments are presented in Table IX. Challenge of normal (nonradiated) mice resulted in death for 64% of these animals; whereas challenge of immunized (nonradiated)

TABLE IX

THIRTY-DAY MORTALITY OF IMMUNIZED AND NONIMMUNIZED MICE CHALLENGED WITH S. TYPHIMURIUM FOLLOWING ACUTE AND CHRONIC WHOLE BODY X-IRRADIATION EXPOSURE

X-irradiation Dose (r)	X-irradiation Exposure	Procedures carried out	Mortality No./total	Mortality %	P values*	Mean day of death for non-survivors
0	-	Normal challenge control	16/25	64		12.2
0	-	Immune challenge control	7/25	28	.05	14.5
300	Single	X-irradiation control	0/28	0		
300	Single	X-irradiation + challenge	25/25	100		11.8
300	Single	Immunization+x-irradiation+challenge	16/25	64	.001	13.6
350	Single	X-irradiation control	5/28	18		12.2
350	Single	X-irradiation + challenge	25/25	100		10.7
350	Single	Immunization + x-irradiation + challenge	18/26	69	.02	14.1
400	Single	X-irradiation control	11/27	41		11.4
400	Single	X-irradiation + challenge	22/25	100		10.3
400	Single	Immunization + x-irradiation + challenge	18/25	72	.02	11.0
450	Single	X-irradiation control	24/27	88		9.2
450	Single	X-irradiation + challenge	25/25	100		8.6
450	Single	Immunization + x-irradiation + challenge	25/25	100		8.2
500	Single	X-irradiation control	27/27	100		8.4
500	Single	X-irradiation + challenge	25/25	100		7.2
500	Single	Immunization + x-irradiation + challenge	25/25	100		7.6
900	Multiple+	X-irradiation control	11/32	35		14.8
900	Multiple	X-irradiation + challenge	25/29	86		10.4
900	Multiple	Immunization + x-irradiation + challenge	2/20	10	.001	13.4

*Probability as calculated by Chi square (X^2) technique. Significant values at the 5% level are underlined.
+See test for x-irradiation exposure schedule.

mice was lethal for only 28%. Challenge of nonimmunized x-irradiated mice resulted in 100% mortality in all groups regardless of the dose of radiation delivered, with the exception of the chronically (multiple dose) exposed animals. The immunizing procedure significantly increased the resistance of mice to infection among animals exposed to as much as LD_{41} x-radiation, but at levels of radiation which resulted in a mortality greater than this, immunization afforded no protection. Chronic (multiple exposure) irradiation, as carried out in this study, resulted in 35% mortality and markedly increased susceptibility to infection (LD_{86}), but failed to break down the actively induced resistance of the mice to subsequent challenge (LD_{10}). Immunization increased the mean day of death for nonsurvivors only when x-irradiation resulted in a mortality of less than 50%.

3. Effect of immunization and/or antibiotic therapy on S. typhimurium infection in x-irradiated mice. Antibiotic therapy has been used to prevent death in animals exposed to various doses of x-irradiation (Genshery et al., 1953; Smith et al., 1953 and Miller et al., 1952). It seemed apparent therefore, that antibiotic therapy in conjunction with preradiation immunization might be utilized to combat postradiation infection. In the present experiment these two methods were employed simultaneously for maximum protection of x-irradiated mice. Adult albino mice were given five 0.1 ml. intraperiteneal injections of vaccine at 5 day intervals with 1 week elapsing between the final injection and radiation exposure. With the exception of the challenge control group, the animals received a single total body dose of 425 r. All mice with the exception of the x-ray control group were challenged by gastric instillation of S. typhimurium on the third postradiation day. Antibiotic therapy was instituted 24 hrs. following challenge.

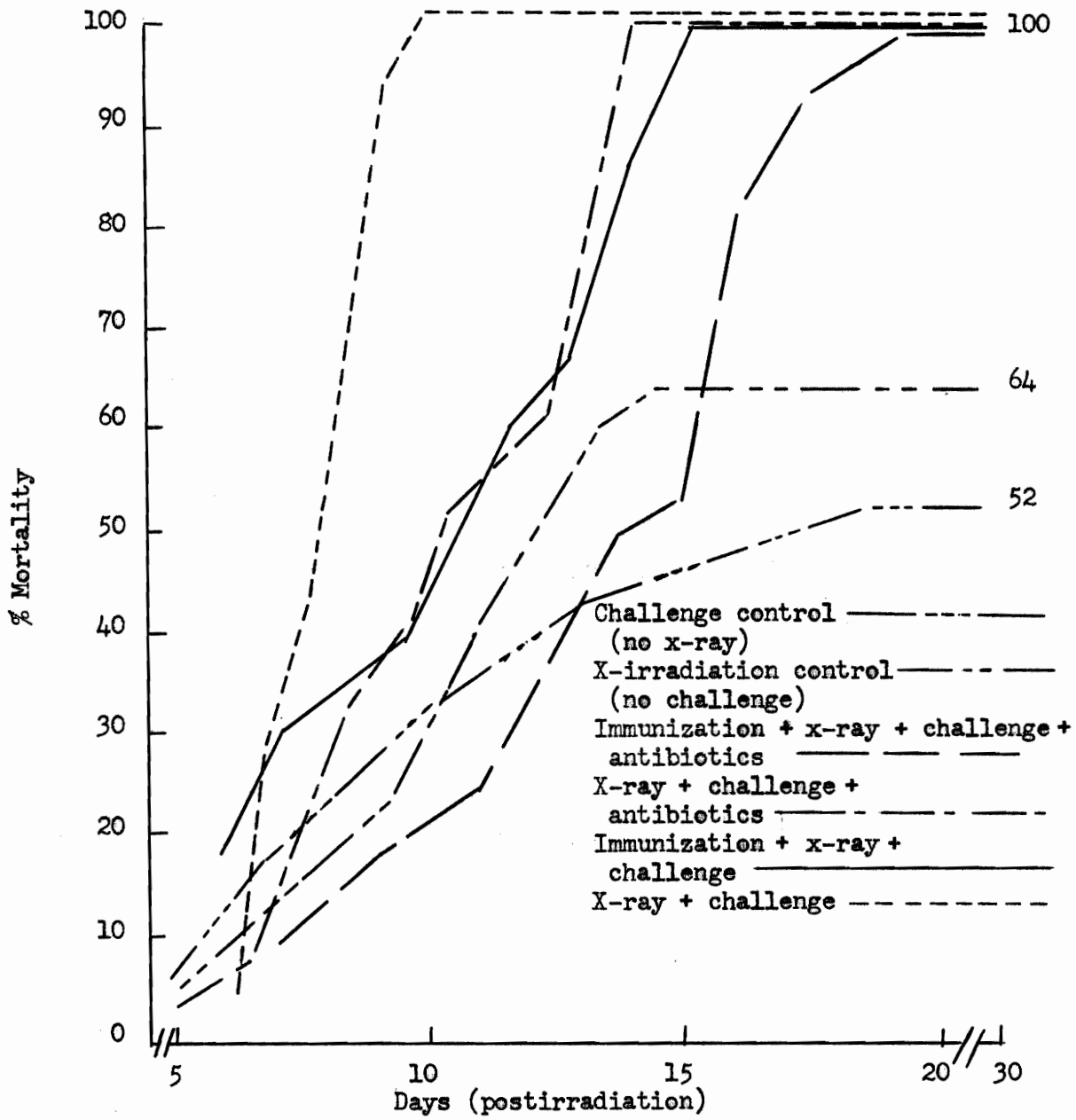

Figure 6

Effect of immunization and/or antibiotic therapy on S. typhimurium infection in x-irradiated mice.

Mice received daily subcutaneous injections of 0.2 ml. of an antibiotic mixture containing 5 mg streptomycin sulfate and 5,000 units of penicillin. Control animals received daily injections of sterile saline. The mortality curves of these groups are shown in Figure 6. One-hundred percent mortality was observed 7 days after challenge of irradiated mice, antibiotic therapy prolonged the LD_{100} to 11 days, immunization to 12 days and the combination of these two procedures to 15 days. The mean time of death for untreated mice was 9.32 days postradiation, for immunized mice 10.84 days postradiation, for antibiotic treated mice 11.12 days and for immunized and antibiotic treated mice 13.6 days.

4. Studies utilizing B. tularense. The opportunity to extend this work to experiments with B. tularense was made available by collaboration with other investigators in this Department. The experimental design employed was similar to that in work with S. typhimurium ; however, the method of immunization employed requires special attention. Immunized groups 11a and 22a (Table 10) consisted of animals which had received three 0.25 ml subcutaneous injections of a "spent medium concentrate"* of B. tularense strain 425 at 2 day intervals with 10 days elapsing between the final injection and subcutaneous challenge of graded doses of the living organisms. Among groups of mice challenged, the number of survivors were as follows:

No. of organisms in challenge injection	16	162	1,620	16,200	162,000
No. of animals surviving challenge	8	10	9	9	5

*Organisms were grown in liquid medium and harvested by centrifugation. The supernated was then concentrated and served as the immunizing material. The author acknowledges the assistance of Dr. Paul S. Nicholes and Mr. Melvin Hatch in the experimental work utilizing B. tularence.

The 41 surviving mice were treated as a homogenous group and divided randomly into groups 11a and 22a.

Groups 33a and 44a were animals which had received no immunizing injections but had survived a subcutaneous challenge of graded doses of B. tularense, strain 4, as follows:

No. of organisms in challenge injection	16	162	1,620	16,200	162,000
No. of animals surviving challenge	8	4	3	5	1

The 21 surviving mice were divided randomly into groups 33a and 44a.

All mice were exposed to a 350 r dose of total body x-irradiation and challenged on the fourth postradiation day. Challenge consisted of gastric instillation of 0.2 ml of a broth culture of B. tularense strain Schu (highly mouse virulent) containing approximately 260 organisms.

The percent mortality results are recorded in Table X. It is seen that x-irradiation increased susceptibility to infection with B. tularense. (Compare groups 22b and 44b with groups 11b and 33b). Immunization, which was effective in increasing the resistance of nonradiated animals, was also effective in protecting x-irradiated animals. (Compare group 11a with 11b and group 22a with 22b). The depression of resistance in immunized animals following x-irradiation, however, is demonstrated by comparison of groups 11a and 22a. Resistance conferred by survival of an infection induced by varied doses of B. tularense was not as complete as that induced by immunization of "spent medium concentrate" and subsequent survival of the induced infection (compare group 22a with 44a). This observation may explain the lack of protection noted in both nonradiated and irradiated animals which were immunized in

TABLE X

EFFECT OF IMMUNIZATION AND X-IRRADIATION (350 r) ON SUSCEPTIBILITY TO INFECTION FOLLOWING ORAL CHALLENGE* WITH B. TULARENCE

Group	Preradiation treatment	X-irradiation	Mortality no/total	Mortality %	P† values
11a	Immunization with "spent medium concentrate" and survival following subsequent challenge	350 r	2/20	65	.05-.02
11b	None	350 r	15/15	100	
22a	Immunization with "spent medium concentrate" and survival following subsequent challenge		5/25	25	.02-.01
22b	None		11/15	73	
33a	Immunization: animals survived living organism challenge	350 r	8/10	80	.30-.20
33b	None	350 r	15/15	100	
44a	Immunization: animals survived living organism challenge		5/10	50	
44b	None		11/15	73	
55	X-irradiation control (no challenge)	350 r	8/20	40	

*Mice challenged 4 days following irradiation exposure with approximately 260 organisms of strain Schu (highly mouse virulent).
†Probability as calculated by Chi square (X^2) technique. Significant values at the 5% level are underlined.

this manner (compare group 33a with 33b and group 44a with 44b).

D. An Attempt to Reduce Mortality of Lethally Radiated Mice by Preradiation Immunization with an Antigenic Extract of Escherichia coli

Bacteremia of enteric origin occurs following exposure to ionizing radiation (LD_{50}) in a high precentage of mice during the second postradiation week. Escherichia coli has been isolated in pure culture with great frequency as a member of the normal enteric flora responsible for death by blood stream invasion (Miller et al., 1950; Genshery et al., 1953 and Vogel et al., 1954). The albino mice used for experimental purposes in this laboratory have yielded E. coli as the bacteremic organism following radiation exposure approximately 35 percent of the time. Smith, et al. (1954) have reported failure to decrease mortality of mice immunized with a heat-killed E. coli vaccine prior to LD_5 irradiation and subsequent challenge with the homologous organism. Observations in this laboratory however, of uniformly high agglutinin titers in mice immunized with somatic "O" antigen prepared by the method of Edwards (1951) stimulated the present effort to determine if specific immunization with comparatively large amounts of this antigen would increase the degree of resistance and reduce mortality following x-radiation by contributing to control of the endogenous infection caused by this organism.

In the present experiment the failure of immunization to increase survival by administration of an antigenic extract of a bacterial species commonly found in the blood of lethally radiated mice is reported.

A group of 35 adult albino mice were immunized by giving five 0.2 ml interperiteneal injections at three day intervals of E. coli vaccine (described in Material and Methods of this Thesis). Thirty days after the final injection of this vaccine mice were paired with 30 non-immune animals and exposed to 400 r of total body x-irradiation. Challenge consisted of the endogeneus invasion by E. coli and the other enteric organisms of the normal intestinal flora. Animals were checked daily for death and cumulative mortalities were recorded. Comparison of the mortality curves recorded in Figure 7 reveals that no difference in 30 day mortalities are present although specific immunization prolonged survival time.

It seems apparent from the results recorded in this section on immunization studies that this procedure can be used as a method for enhancement of resistance of x-irradiated animals exposed to levels not exceeding the LD_{50} when challenge is administered by method simulating natural routes of infection, i.e., either respiratory or gastrointestinal. With this fact clearly established, effort has been made to determine the basic protective mechanisms involved in the observed specific bacterial resistance following immunization of irradiated animals.

II. SUPPRESSION OF SERUM BACTERICIDAL ACTIVITY AND ITS RELATION TO POSTIRRADIATION INFECTION

Observation first made in this laboratory (Marcus and Donaldson, 1954) have demonstrated that suppression of serum bactericidal activity occurs following whole body ionizing irradiation. It was suggested that the depressed bactericidal activity of serum following irradiation

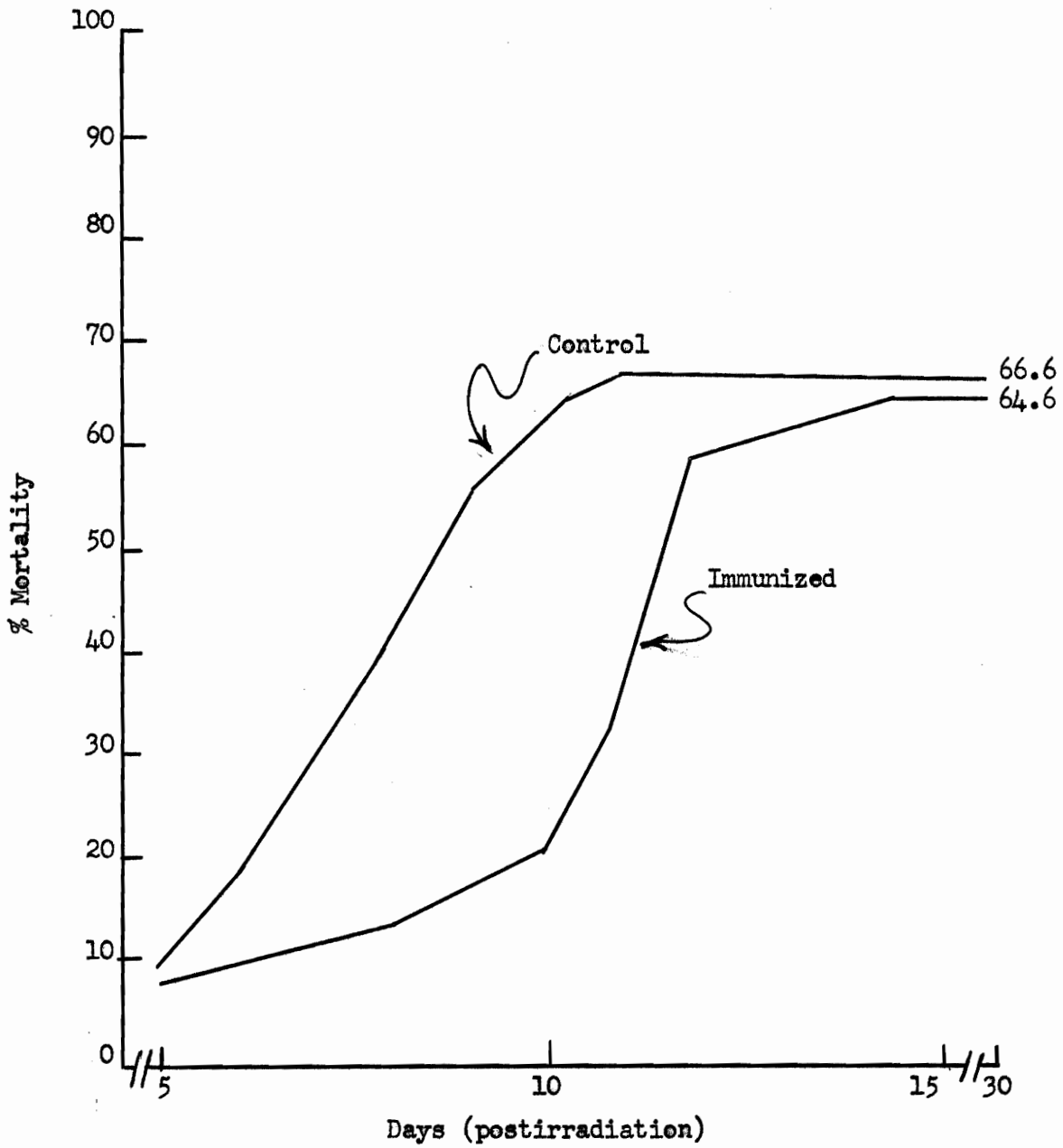

Figure 7

Effect of immunization with an antigen extract of E. coli on mortality of x-irradiated mice.

might be of critical significance. Experiments have been carried out to determine the effect specific immunization might have upon the bactericidal activity of sera from normal and irradiated rats. Furthermore, effort has been made to determine the relationship of decreased serum bactericidal activity and the onset of bacteremia in irradiated animals.

A. The Effect of Immunization and X-irradiation on the Bactericidal Activity of Serum

Albino rats were immunized against B. subtilis by three 0.5 ml intraperitoneal injections of heat killed vaccine on alternate days. One month later a second series of 3 injections was given at 10 day intervals. Sixty days after the last immunization injection these animals were exposed to 600 r of total body x-irradiation. A second group of rats were immunized against S. typhimurium with three 0.5 ml intraperitoneal injections at 14 day intervals with 2 weeks elapsing between the final injection and exposure to 800 r x-irradiation. On the eighth postirradiation day blood was drawn by cardiac puncture from irradiated and non-irradiated animals, the serum separated and bactericidal tests performed. Since rat serum is highly bactericidal, it was decided that if the serum dilutions employed were designed to include that dilution at which bactericidal action was observed to end, that is, the bactericidal titer, difference among sera from immunized, immunized irradiated, normal and normal irradiated animals might be more readily detected.

The results obtained in these studies utilized this design and are recorded in Tables XI and XII. It can be seen that there is a loss of

TABLE XI

EFFECT OF X-IRRADIATION AND IMMUNIZATION UPON THE BACTERICIDAL ACTIVITY OF RAT SERUM AGAINST B. SUBTILIS

Groups of rats		X-irradiation received (r)	Bacteria / ml.* Serum dilution 1:20	1:40	1:80	Anti- B. subtilis agglutinin titer (reciprocal)
IMMUNE RATS	1	None	0	11	39	512
	2	None	1	66	34	256
	3	None	5	1	340	256
	4	600	1935	1672	2016	256
	5	600	380	2812	2600	256
	6	600	2380	2010	2880	512
NORMAL RATS	1	None	0	1660	576	16
	2	None	1	15	42	16
	3	None	0	2	82	8
	4	600	2880	3056	2912	8
	5	600	2944	3680	3500	8
	6	600	2400	2400	1480	16

*All figures x 10^5 for total number of organisms per ml. Prior to the 2 hour incubation serum contained an initial concentration of approximately 106 x 10^5 organisms/ml.

TABLE XII

EFFECT OF X-IRRADIATION AND IMMUNIZATION UPON THE BACTERICIDAL ACTIVITY OF RAT SERUM AGAINST S. TYPHIMURIUM

Groups of rats		X-irradiation received (r)	Bacteria / ml* Serum dilution 1:10	1:20	1:40	Anti-S. typhimurium "O" agglutinin titers (reciprocal)
IMMUNE RATS	1	None	240	1830	3640	80
	2	None	190	1520	3410	160
	3	800	3240	2910	3140	80
	4	800	3560	3290	2950	80
NORMAL RATS	1	None	420	1630	3520	10
	2	None	87	1790	3840	10
	3	800	2980	3740	3200	10
	4	800	3340	3920	2860	10

*Prior to two hour incubation each serum contained an initial concentration of approximately 3150 organisms/ml.

serum bactericidal activity of the sera of both immunized irradiated and normal (non-immunized) irradiated rats. There appears to be no significant difference in the bactericidal activity of the irradiated sera from either normal or immunized animals. Bactericidal activity is evidently at a maximum in nonimmunized animals since no difference exists between the bactericidal activity of non-irradiated normal and non-irradiated immune sera. It should be noted that x-irradiation apparently had no deleterious effect on preformed antibody since agglutinin titers of x-irradiated animals are comparable to those of non-irradiated rats. From these results it can be concluded that immunization did not enhance the bactericidal activity of sera from either normal (non-irradiated) or irradiated rats and in no way reversed the depressed bactericidal activity of sera following irradiation exposure.

B. Relationship of Onset of Bacteremia in the X-irradiated Animal to Decreased Serum Bactericidal Activity: A Comparative Study

Since death following exposure to moderate dose of x-irradiation (LD_{50}) is attributable to overwhelming infection of endogenous origin, investigations have been extended to determine if the depression of serum bactericidal activity can be temporally related to, or is merely coincident with the onset of bacteremia in the irradiated animal. The experimental design consisted of exposing animals to x-irradiation and then withdrawing blood by cardiac puncture and testing for depression of bactericidal activity and the presence of bacteremia at given intervals from the middle of the first postirradiation week to the end of the second postirradiation week. All animals were followed individually.

1. Using the rat as the experimental animal. Adult albino rats were exposed to 600 r of total body x-irradiation and the loss of serum bactericidal activity and the presence of bacteremia were tested for on the third, fifth, seventh, ninth, eleventh and fourteenth post-irradiation day. The results obtained are present in Table XIII. These results demonstrate an apparent lack of correlation between loss of serum bactericidal activity and the onset of a bacteremia in irradiated rats. Loss of serum bactericidal activity was not prominent until the ninth postirradiation day; whereas bacteremia occurred in some instances prior to loss of serum bactericidal activity (third, fifth and seventh postirradiation day); furthermore, some animals exhibited a bacteremia while bactericidal activity was apparently normal and all animals which exhibited depressed bactericidal activity did not develop bacteremia. Such results indicated that loss of serum bactericidal activity may be merely coincident with, rather than related to the onset of bacteremia in irradiated rats.

2. Using the rabbit as the experimental animal. Table XIV demonstrates the failure of rabbits to develop bacteremia following x-irradiation (600 r) although bactericidal activity is markedly impaired. The depression of serum bactericidal activity is noted in one of nine rabbits on the seventh postirradiation day, on the ninth postirradiation day 7 of 10 animals showed depression of serum bactericidal activity and on the eleventh postirradiation day serum from all rabbits exhibited loss of bactericidal activity. It can be seen that during this same period of time that not a single rabbit developed demonstrable bacteremia.

3. Using mice as the experimental animal. The mouse is a unique laboratory animal in that serum from normal (non-irradiated) mice fails to

TABLE XIII

THE LACK OF CORRELATION BETWEEN THE LOSS OF SERUM BACTERICIDAL ACTION AND THE ONSET OF BACTEREMIA IN X-IRRADIATED (600 r) RATS

Observations	Days (postirradiation) 3rd	5th	7th	9th	11th	14th	Controls (no x-ray)
Loss of serum bactericidal activity	0/3	1/10	0/9	6/7	6/7	4/6	0/10
Development of bacteremia	1/8	2/10	3/9	2/7	3/7	0/6	0/10

TABLE XIV

THE FAILURE OF RABBITS TO DEVELOP BACTEREMIA FOLLOWING X-IRRADIATION (600 r) ALTHOUGH BACTERICIDAL ACTIVITY OF SERUM IS MARKEDLY IMPAIRED

Observations	Days (postirradiation) 3rd	5th	7th	9th	11th	13th	Controls (no x-ray)
Loss of serum bactericidal activity	0/10	0/10	1/9	7/10	7/7	7/7	0/10
Development of bacteremia	0/10	0/10	0/9	0/10	0/7	0/7	0/10

exhibit bactericidal activity; Apparently this host defense mechanism, common to other animals, is of little significance in mice. Table XV records the time of onset of bacteremia in the irradiated (LD_{48}) mouse. Bacteremia begins and extends through the second postirradiation week and characteristically leads to a fatal termination.

Since the mechanism(s) responsible for the prevention of overwhelming bacteremia in rabbits and its occurrence in rats and mice cannot be explained on the basis of the depression of serum bactericidal activity and since immunization could not reverse the depressed bactericidal activity, effort was extended to investigate the role of antibody in enhanced resistance of immunized irradiated animals.

III. THE CONTRIBUTING ROLE OF PREFORMED ANTIBODY IN THE RESISTANCE OF THE IRRADIATED ANIMAL

A. The Effect of X-irradiation on Preformed Antibody and Its Role in the Protection of X-irradiated Mice

A conclusive literature exists (Literature Review, section II, A) to support the hypothesis that x-irradiation does not alter preformed antibody when the level of irradiation employed does not exceed an LD_{50} for the experimental animal. The following experiments were conducted to determine if higher doses of irradiation (LD_{100}) might alter the serological activity of preformed antibody and to clarify the role that antibody plays in the protection of the x-irradiated animal.

1. Effect of various doses of x-irradiation on induced hemagglutinin titers of the rat. The animals used in this experiment were male albino rats weighing between 200 and 250 gms and were immunized prior

TABLE XV

DEVELOPMENT OF BACTEREMIA

IN X-IRRADIATED (450 r) MICE

Observations	Days (postirradiation)					
	5th	7th	9th	11th	13th	15th
Development of bacteremia	1/25	3/23	3/17	1/13	1/12	0/11

to x-irradiation. The immunization procedure consisted of three intraperitoneal injections of 0.5 ml of a 2.0% suspension of washed human type A erythrocytes given at three day intervals with three weeks elapsing between the final injection and irradiation exposure. Each group consisted of ten animals. Group I was exposed to 300 r x-irradiation (sublethal); Group II, 500 r (midlethal); Group III, 700 r (LD_{100}). Group IV received no irradiation and served as the immunized nonirradiated control.

Blood was collected by means of cardiac puncture three days prior to x-irradiation and 3, 7, 11, and 15 days postradiation. Serum was separated immediately and agglutinin titrations carried out.

The results recorded in Figure 8 indicate that neither the titers nor the serological activity of induced agglutinins for human type A erythrocytes were significantly altered by exposure to 300 r of total body x-irradiation 3, 7, 11 or 15 days following irradiation exposure. Similar results are noted at the 500 r level of exposure. However, on the eleventh post-irradiation day only 5 serums were tested and on the fifteenth day postirradiation only 2 serums; the other animals had died. This was presumably due to the cumulative effects of x-irradiation, cardiac puncture and decreased blood volume. Similar results were recorded at the 700 r level of x-irradiation; however, serums were only available 3 and 7 days postirradiation. Agglutinin titers from irradiated animals did not vary from control values (nonirradiated) within the limits of the experimental error for the serological technique utilized.

2. Effect of supralethal irradiation on the protective capacity of preformed antibody. Since x-irradiation, even in highly lethal doses

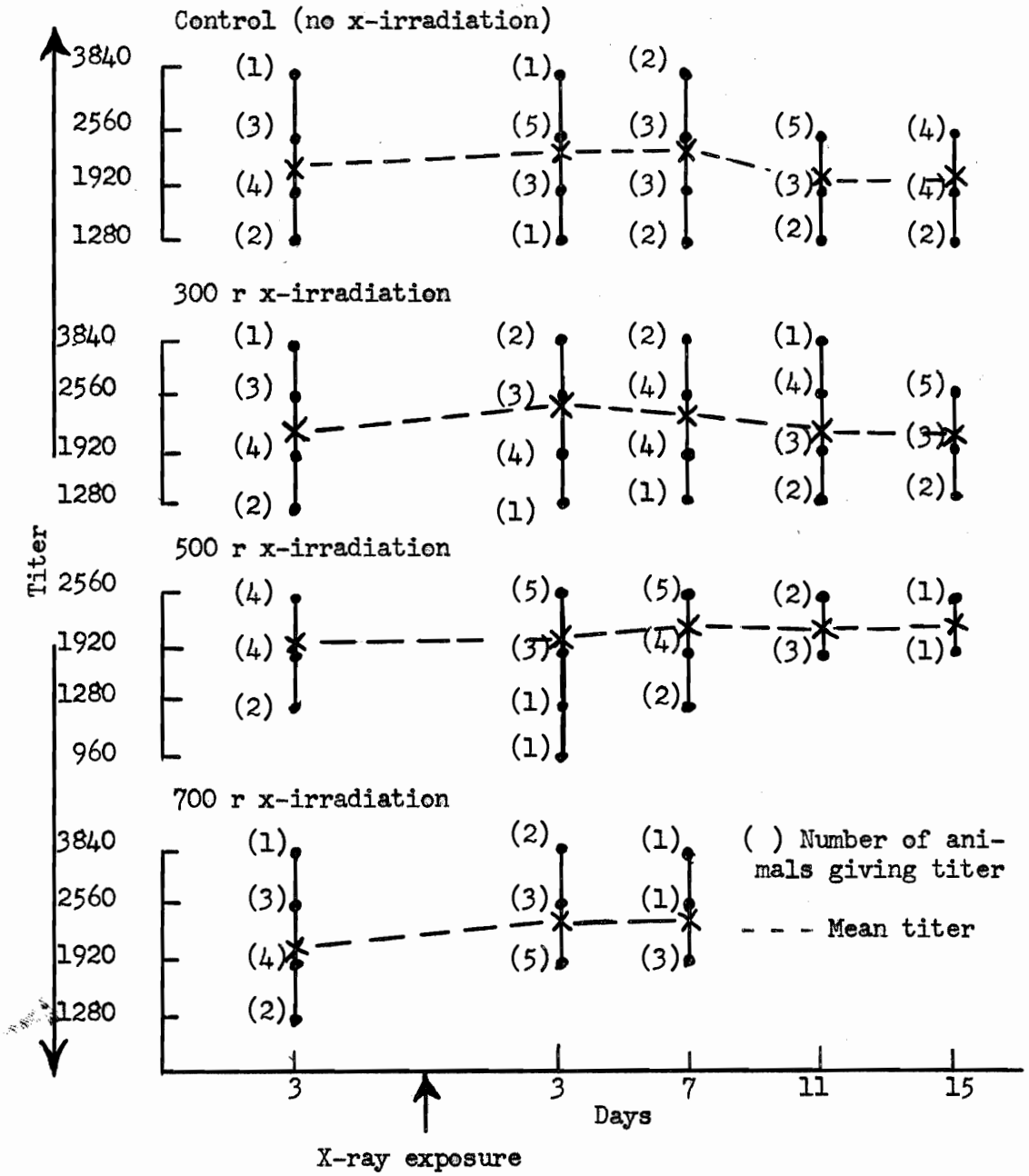

Figure 8

Anti-human type A red blood cell agglutinin titers of serums from immunized rats.

(LD_{100}) had no apparent effect on the serological activity of antibody *in vitro*, it was deemed desirable to determine the effect irradiation might have on the protective capacity of antibody *in vivo*. The hypothesis examined was that passive immunization with serum of specifically immunized, and later lethally irradiated animals, would afford the same degree of protection against infection following challenge with the homologous organism as would serum of specifically immunized nonirradiated animals. To test this hypothesis the following definitive experiment was carried out after preliminary work suggested the feasibility of the experimental design.

Mice were immunized with three 0.2 ml intraperitoneal injections with *K. pneumoniae* vaccine at five day intervals. Two and one half weeks after mice had received their last injection, these animals were divided into two groups and one group was exposed to a total body dose of x-irradiation of 600 r. Two days later animals of both groups were sacrificed by decapitation; blood was collected aseptically, serum separated and, this same day, normal (nonirradiated) test animals were passively immunized by intraperitoneal injection with 0.5 ml of these sera. Forty-eight hours after groups of mice received normal antibody, antibody from x-irradiated mice, or sterile saline (control group), these animals were challenged intraperitoneally with approximately 350 *K. pneumoniae* organisms. The thirty day mortality results of these groups are shown in Table XVI. It is seen that supralethal irradiation had no effect on the protective capacity of preformed antibody. Antibody from lethally irradiated mice was just as effective in preventing death as was antibody from non-irradiated mice.

TABLE XVI

PROTECTIVE CAPACITY OF SERUMS OBTAINED FROM IMMUNIZED AND NONIMMUNIZED X-IRRADIATED (600 r) MICE AGAINST A CHALLENGE INFECTION OF K. PNEUMONIAE

Group	Treatment prior to challenge*	30 day mortality	P
A	Serums from immunized mice	7/30 - 23%	.001
B	Serums from x-irradiated immunized mice	5/28 - 18%	.001
C	Normal saline	31/35- 88%	-

*Intraperitoneal injection of 0.5 ml of pooled serum (titer 1:64) 48 hours prior to intraperitoneal challenge.
Probability when compared with saline control as calculated by Chi square (x^2) technique.

3. The role of preformed antibody in the protection of the irradiated mouse. A final consideration in this study was to determine the role antibody played in protection of the irradiated mouse. In order to divorce any effect that active immunization might have on enhancement of cellular mechanisms of defense, x-irradiated mice were passively immunized and then infected with K. pneumoniae. These animals received 0.5 ml of pooled homologous serum (agglutination titer 1:64) from actively immunized mice forty-eight hours prior to challenge. For comparative purposes controls included actively immunized x-irradiated mice and nonimmunized x-irradiated mice. All mice, with the exception of "x-irradiation controls" were challenged on the sixth postirradiation day. Challenge consisted of a 0.2 ml. intraperitoneal injection of a broth culture of K. pneumoniae containing approximately 600 organisms. Figure 9 summarizes the treatment administered and records the thirty-day mortality results. It is seen that for nonirradiated mice or animals which had been exposed to either 350 or 400 r of total body x-irradiation that passive immunization was as effective in protecting mice as the active process. In the group exposed to 425 r, however, passive immunization failed to significantly reduce mortality, whereas active immunization did significantly reduce mortality. All animals which had been exposed to a 450 r dose of x-irradiation were uniformly susceptible to the challenge infection. At this dose level neither active nor passive immunization afforded protection against the induced infection.

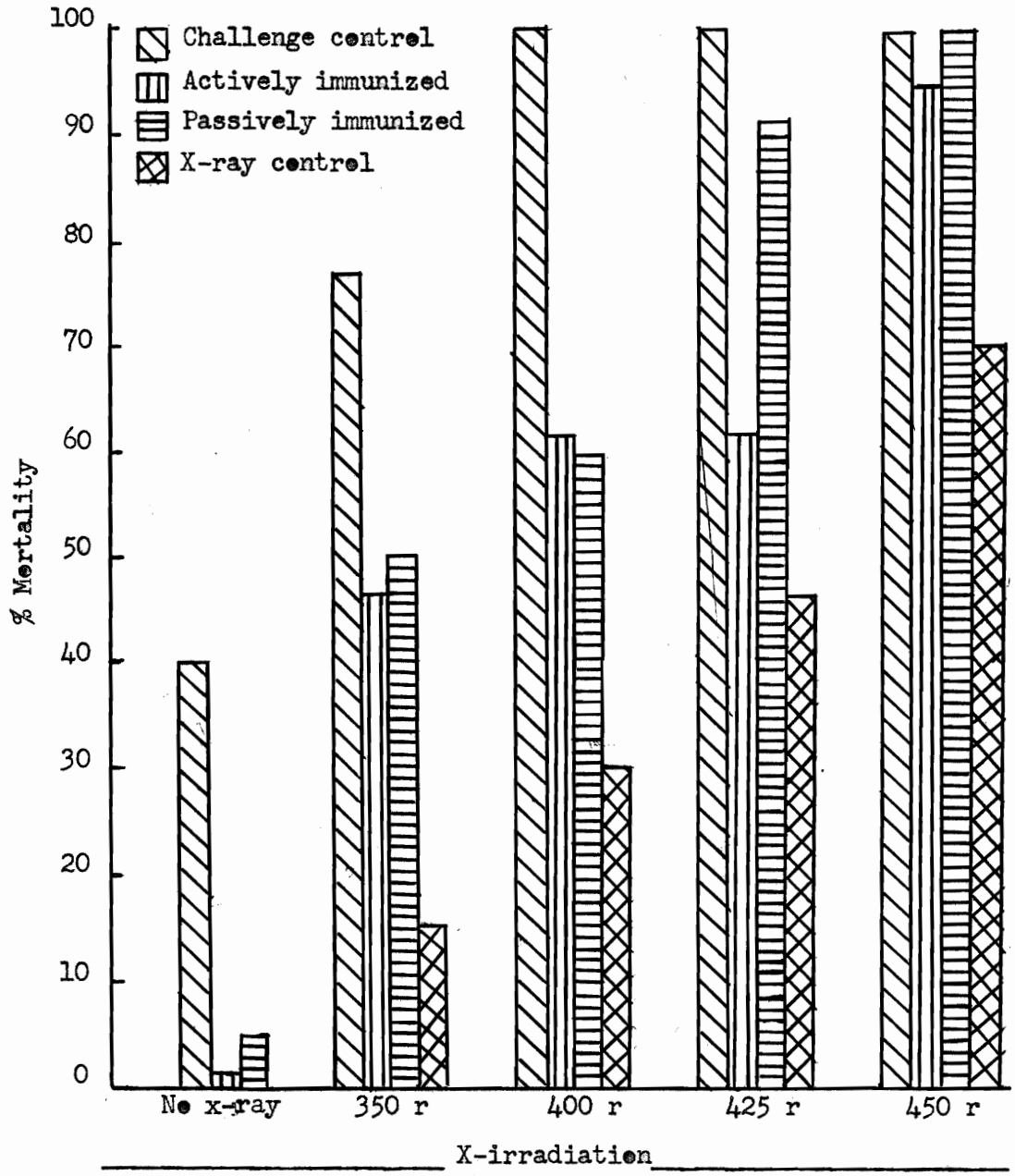

Figure 9

Thirty-day mortality results of actively or passively immunized x-irradiated mice subsequently infected with K. pneumoniae.

IV. THE RELATIONSHIP OF CELLULAR FACTORS TO ANTIBACTERIAL RESISTANCE IN THE X-IRRADIATED HOST

Although it is currently doctrinal to describe specific resistance against infectious disease in terms of antibody response, experiments with irradiated animals have yielded an abundance of indirect evidence which dramatically illustrates that cellular integrity must be maintained if the irradiated host is to remain free of infectious disease. The hypothesis is suggested that it is this damage of cellular mechanisms which results in the collapse of antibacterial resistance. The following section is devoted to an experimental evaluation of this hypothesis.

A. Enhanced Survival of Irradiated Mice Following Administration or Stimulation of Peritoneal Leukocytes

It has been demonstrated conclusively that administration of haemopoietic tissue preparations (suspensions of bone marrow or splenic tissue) can modify the lethal effects of whole body ionizing radiation (Lorenz et al., 1951; Jacobson et al., 1951; Silverman, et al., 1956, and Trenton, 1956). Only recently has it been established that intact viable haemopoietic cells are necessary to provide cellular precursors which are capable, at least temporarily, of maintaining the host by cellular proliferation and repopulation of depleted haemopoietic tissue pending functional restoration of haemotopoiesis within the host (Ford et al., 1956; Lindsley, et al., 1955; Nowell et al., 1956; Bos et al., 1956, and Smith et al., 1957). The fundamental problem concerning the nature of the protective activity of the haemopoietic tissue still seeks clarification. The present preliminary experiments report the

ability of peritoneal leukocytes (phagocytes) to afford protection to x-irradiated mice, thereby suggesting a rational mechanism of action for postirradiation protection following injection of haemotopietic tissue at radiation levels where infection plays a prominent role.

1. Challenge experiments. As cellular elements are critically reduced and functionally impaired following irradiation injury it was suggested that if the remaining cells could be mobilized and then localized at the site of challenge mortality could be diminished. A cellular infiltration was induced in the peritoneum of irradiated mice by injection of 0.5 ml of a 0.01% glycogen saline solution. These animals were challenged 24 hours later, on the sixth postirradiation day, by intraperitoneal injection of K. pneumoniae. Table XVII presents the results of the first experiment. It can be seen that localization of phagocytic cells at the site of challenge reduced mortality of these animals.

A second experiment was conducted employing the same experimental design, but with alteration of certain experimental factors. The radiation level was increased from 400 to 450 r. Animals were challenged 48 hours rather than 24 hours after glycogen injection, as in the preceding experiment. In addition, peritoneal leukocytes collected from donor mice, were injected into irradiated recipients and then challenged with K. pneumoniae. It can be seen from Table XVIII that mobilization of the host phagocytic system and localization at the site of challenge by i.p. injection of glycogen, again afforded some protection to irradiated animals although at this radiation level (450 r) considerable cellular damage must exist. Contrary to these findings, homologous peritoneal leukocytes afforded no protection. Suggested reasons for this failure will be pursued in the discussion of these experiments.

TABLE XVII

MORTALITY OF X-IRRADIATED (400 r) MICE

RECEIVING AN INTRAPERITONEAL INJECTION OF GLYCOGEN

24 HOURS PRIOR TO INTRAPERITONEAL CHALLENGE WITH *K. PNEUMONIAE*

Group	Postirradiation treatment	Mortality ratio	%
I	0.5 ml of 0.01% glycogen injected 24 hours prior to challenge	12/24	50
II	0.5 ml of saline injected 24 hours prior to challenge	18/23	78
III	X-ray control (no challenge)	7/25	28

TABLE XVIII

MORTALITY OF X-IRRADIATED (450 r) MICE TREATED WITH GLYCOGEN OR PERITONEAL LEUKOCYTES PRIOR TO INTRAPERITONEAL CHALLENGE WITH *K. PNEUMONIAE*

Group	Postirradiation treatment	Mortality ratio	%
I	Intraperitoneal injection of 0.5 ml of 0.01% glycogen 48 hrs. prior to challenge	8/15	53
II	Intraperitoneal injection of 0.5 ml of saline 48 hrs. prior to challenge	12/16	75
III	Intraperitoneal injection of peritoneal leukocytes (16×10^6) 48 hrs. prior to challenge	12/15	80
IV	X-ray control (no challenge)	7/16	44

In a final effort the irradiation level was increased to 500 r. Table XVIV records the mortality results. The 30 day mortality of x-irradiated non-challenged mice (x-ray control) was 72%. Challenge was 100% fatal for groups of animals that received saline or homologous leukocytes, whereas injection of glycogen again reduced mortality, affording slight protection to the mice. From these and preceding experiments it has been demonstrated that a direct relationship exists between the irradiation level and the percent mortality. The present experiments contributes evidence to the hypothesis that it is damage of the cellular mechanisms that result in collapse of antibacterial resistance. In these experiments mortality can be related to the failure of the animal to initiate an adequate cellular response.

2. Attempts to reduce mortality of non-challenged irradiated mice by injection of peritoneal leukocytes. It was hypothesized that injection of actively phagocytic peritoneal leukocytes into irradiated animals might augment, through their phagocytic capacity, the declining resistance of the irradiated recipient. Harvesting homologous peritoneal leukocytes from donor mice, necessitated sacrifice of large numbers of normal animals. With an awareness of the additional problem interposed, more readily available heterologous leukocytes from rats or rabbits were utilized in preliminary experiments.

In the first experiment a single intraperitoneal injection of heterologous leukocytes (rat or rabbit) were given mice immediately following x-irradiation or on the third postirradiation day. It can be seen from Figure 10, that a single injection of either rat or rabbit leukocytes given at these postirradiation times failed to significantly reduce mortality.

TABLE XVIV

MORTALITY OF X-IRRADIATED (500 r) MICE TREATED WITH GLYCOGEN OR PERITONEAL LEUKOCYTES PRIOR TO INTRAPERITONEAL CHALLENGE WITH *K. PNEUMONIAE*

Group	Postirradiation treatment	Mortality ratio	%
I	Intraperitoneal injection of 0.5 ml of 0.01% glycogen 24 hrs. prior to challenge	22/25	88
II	Intraperitoneal injection of 0.5 ml of saline 24 hrs. prior to challenge	24/24	100
III	Intraperitoneal injection of peritoneal leukocytes (17×10^6) 24 hrs. prior to challenge	22/22	100
IV	X-ray control (no challenge)	18/25	72

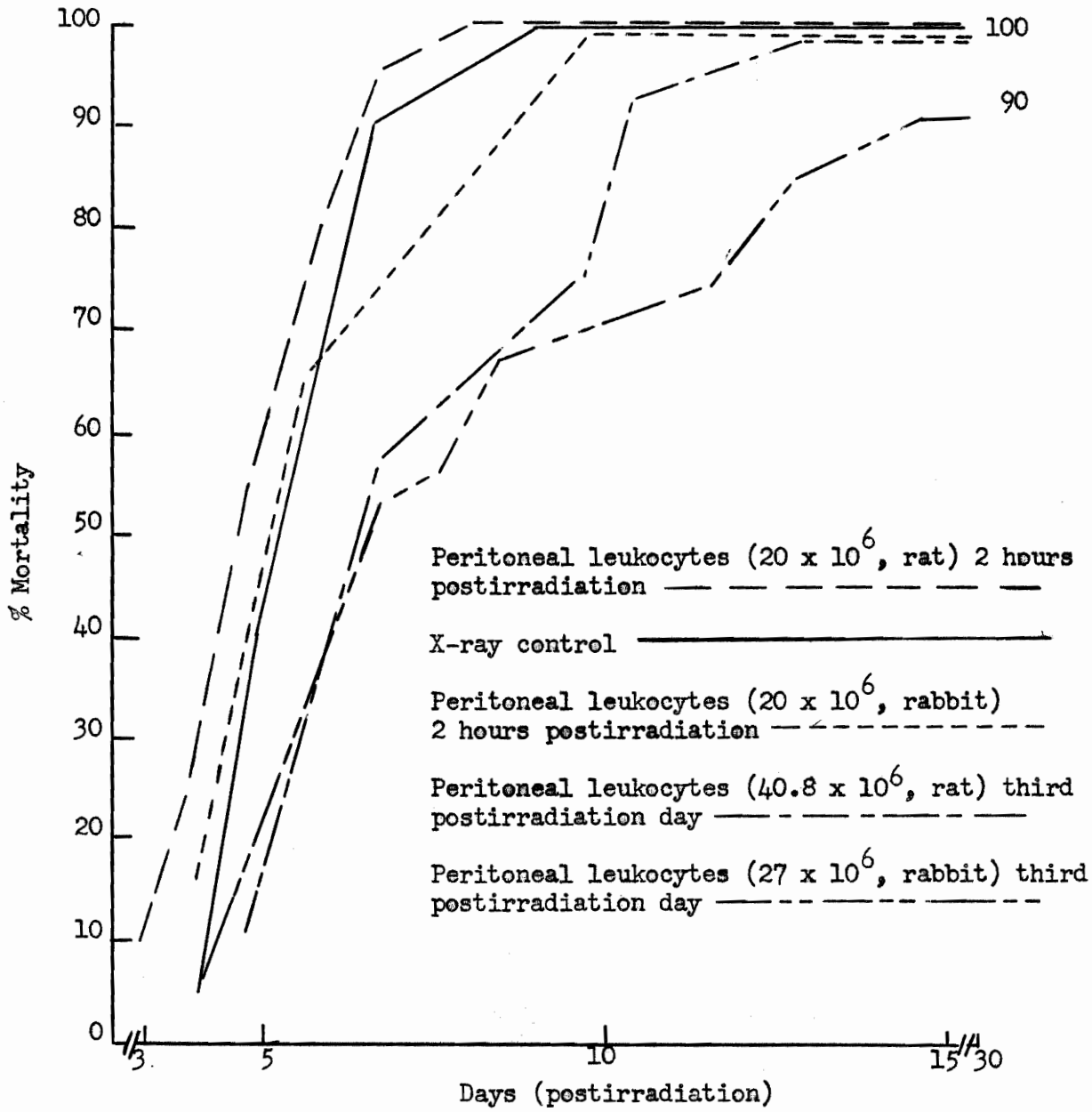

Figure 10

Failure of a single injection of peritoneal leukocytes to afford protection to x-irradiated mice.

Succeeding experiments demonstrated that a single injection of comparatively large numbers of these cells, as many as 85 x 10^6, failed to alter mortality when administered on any given day in the first postirradiation week. Furthermore, a single injection of cells obtained from exudates induced 24 or 72 hours prior to collection, thereby giving differences in cellular composition, failed to afford protection. In twenty-four exudates the predominant cell type was the polymorphonuclear leukocytes whereas in 72 hour exudates monocytes and lymphocytes predominated.

Attempts were therefore made to determine if two injections at critical postirradiation periods would afford protection to the x-irradiated recipients. Results of such experiments are presented in Figures 11 and 12. From Figure 11 it can be seen that mice which were exposed to 450 r (LD_{84}) and then received cellular suspensions intraperitoneally on the sixth and eighth postirradiation day exhibited reduced mortalities, whereas animals which received leukocytes on the fourth and sixth postirradiation day showed no increase in resistance. Undoubtedly, the time of administration is important and must correspond to that period when the normal host defenses must be augmented if survival is to result. Since the life span of the injected leukocytes is relatively short, approximately 24-48 hours, daily injections of functional cells may yield maximum survival. However, continuous injection of large numbers of these cells may impair survival as death and dissolution of these cells can interpose additional stress to the already over-taxed phagocytic system. The significant preliminary finding, delineated in Figures 11 and 12 is that some protection is afforded mice which received only leukocytes (phagocytic cells) independent of other haemopoietic elements.

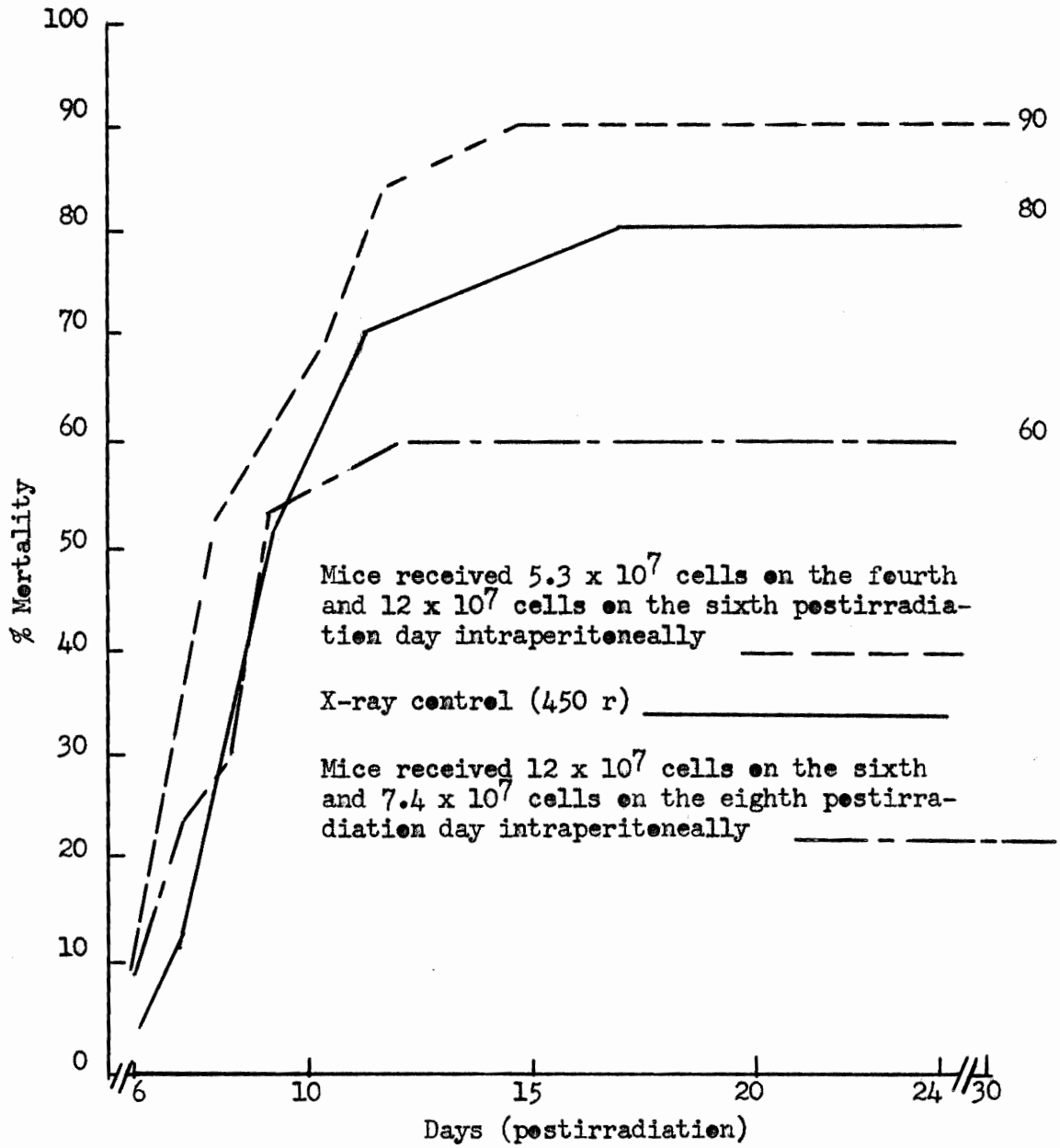

Figure 11

Survival of x-irradiated (450 r) mice following administration of peritoneal leukocytes on the sixth and eighth postirradiation day.

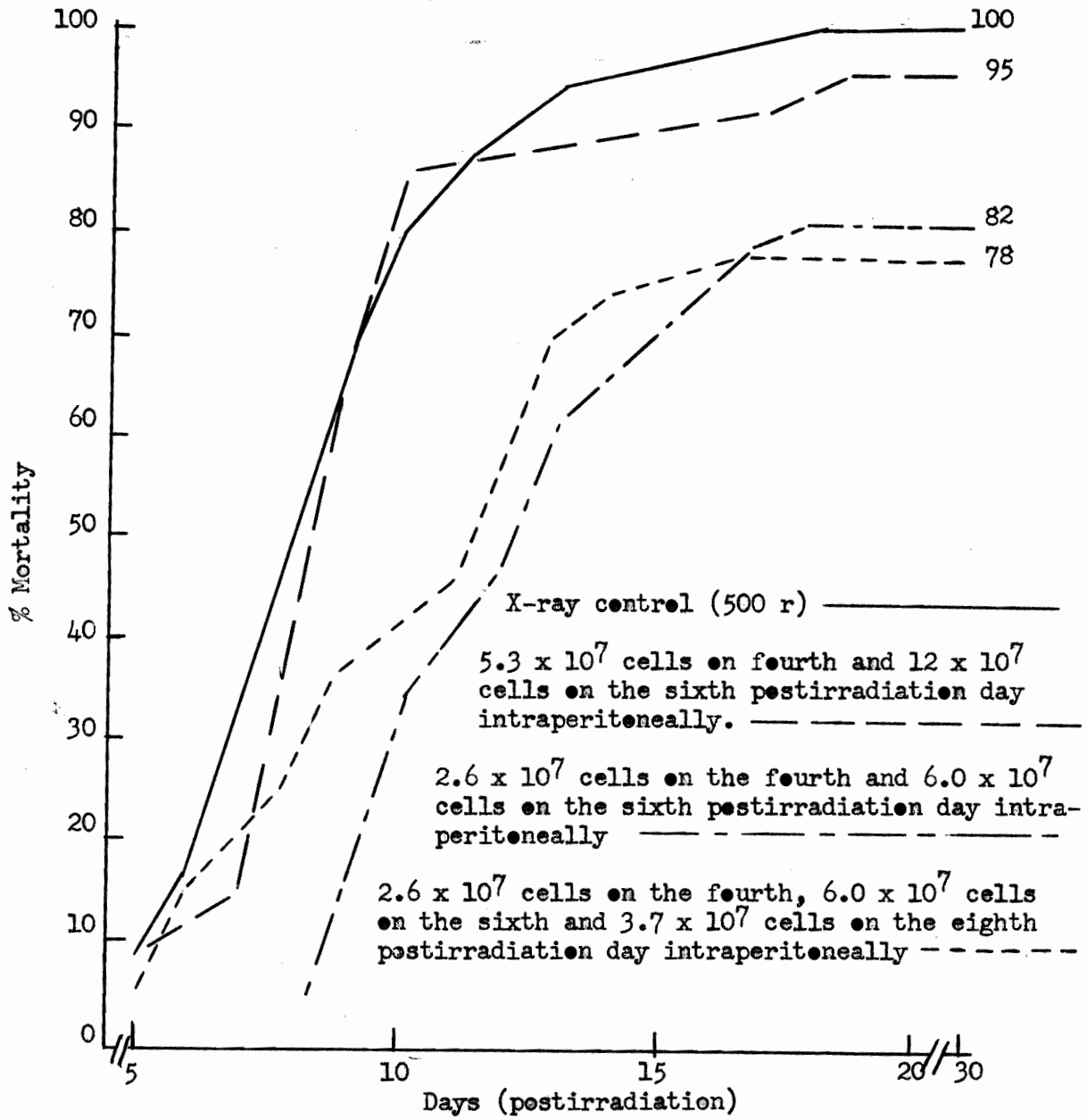

Figure 12

Survival of x-irradiated (500 r) mice receiving injections of peritoneal leukocytes on the fourth and sixth postirradiation day.

B. Enhancement of the Functional Capacity of Cellular Factors in Resistance Following Immunization Independent of Humoral Antibody

The hypothesis first advanced by Metchnikoff (1905) that phagocytic cells of actively immunized animals may be endowed, as a result of the immunizing procedure, with enhanced phagocytic and digestive capacities independent of humoral antibody, has received little attention in the past. More recently, evidence has accumulated to suggest that the cellular elements of animals immunized against the etiological agents of certain chronic infections (tuberculosis, histoplasmosis, brucellosis) have enhanced capacities to destroy phagocytized organisms without regard to the presence of antibody. Effort has been extended to determine if phagocytic cells might exhibit a similar response to acute infections. Both in vitro and in vivo studies have been carried out.

1. In vitro studies. These experiments were conducted using the Warburg apparatus. The working hypothesis was suggested that changes in oxygen uptake could be correlated with death or proliferation of ingested microorganisms in phagocytic cells. Plate counts made on aliquots taken from the reaction flasks following incubation suggested validity for this assumption.

Cellular exudates for these experiments were harvested from normal and immunized rabbits as previously described. The cell suspensions were adjusted to equal concentrations following hemocytometer counts for use in comparative studies. Bacterial cells were harvested from a 24 hour tryptose phosphate broth culture, washed, suspended in the same substrate and standardized in a Klett Summerson colorimeter.

Bacteria were mixed directly with phagocytic cells in the reaction flasks and after equilibration oxygen uptake was measured.

Figure 13 shows the oxygen uptake of Klebsiella pneumoniae when incubated with peritoneal phagocytes from normal or immunized rabbits. Line A represents the bacterial control and gives the respiratory rate of K. pneumoniae under the conditions of the experiment. Line B shows the change in oxygen uptake when bacteria were incubated with normal phagocytes. Line C is a similar system in which phagocytic cells obtained from immunized animals were used, and lines D and E represent the respiratory rate of the phagocytic cells in the absence of bacteria. It can be seen that phagocytes from immunized animals (line C) dramatically inhibited oxygen uptake, whereas this ability was of decreased magnitude and only transitory for phagocytic cells obtained from normal animals. Plate counts at the termination of the experiment showed a marked reduction in the number of viable organisms in the reaction flasks containing "immune" phagocytes.

Figure 14 represents a similar study using Salmonella typhimurium as the test organism. Again, it can be seen that phagocytic cells from immunized animals have an enhanced capacity for destruction of bacteria as measured by inhibition of oxygen uptake. In both experiments all efforts to demonstrate the presence of intracellular or extracellular antibody by agglutination techniques proved negative. In an effort to extend these results, in vivo experiments were carried out.

2. In vivo studies. Peritoneal exudates were induced in normal and immunized mice. Cellular exudates were harvested, washed, quantitated and immediately injected intraperitoneally into groups of recipient

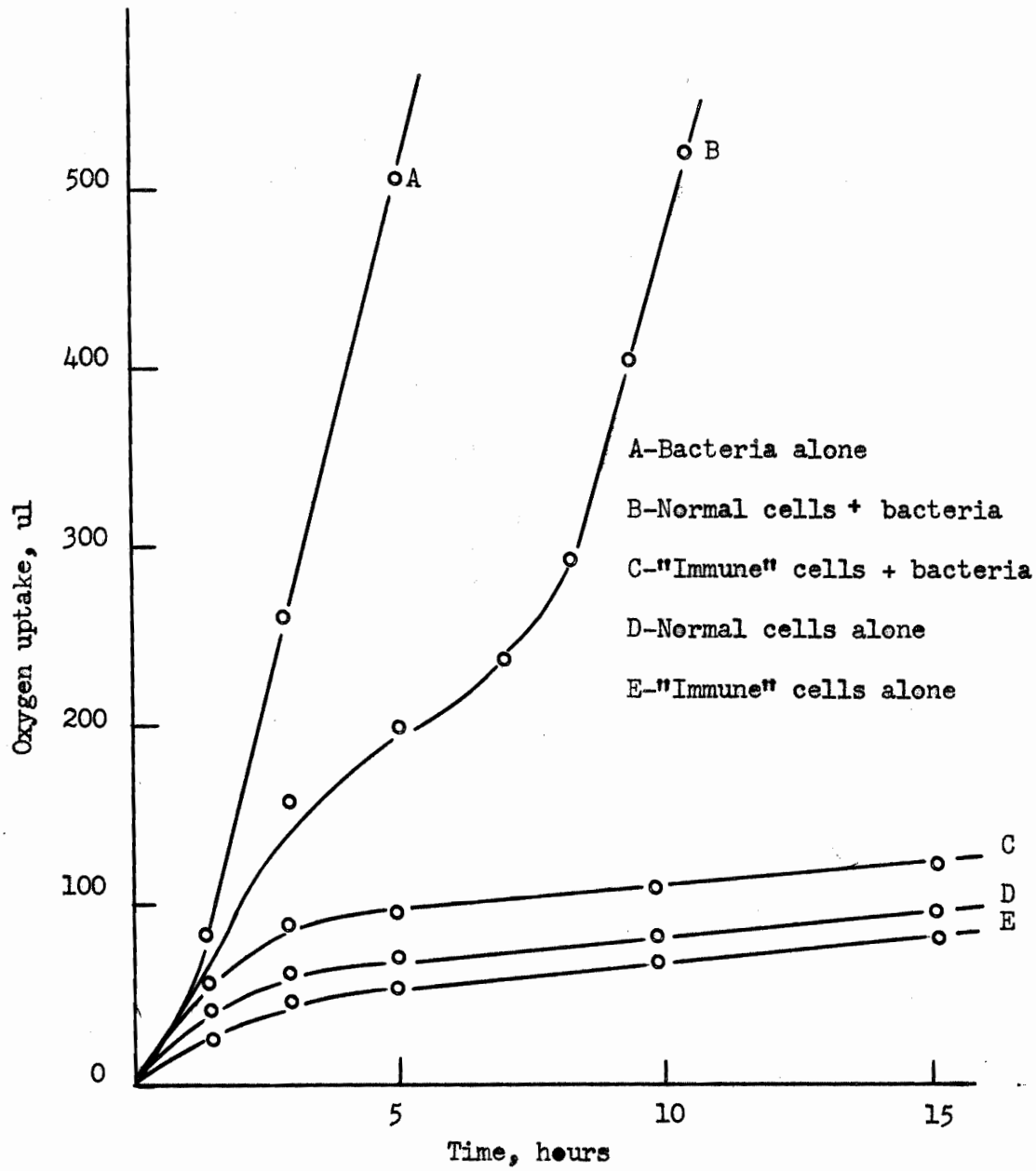

Figure 13

Oxygen uptake of K. pneumoniae when incubated with normal and "immune" rabbit phagocytes.

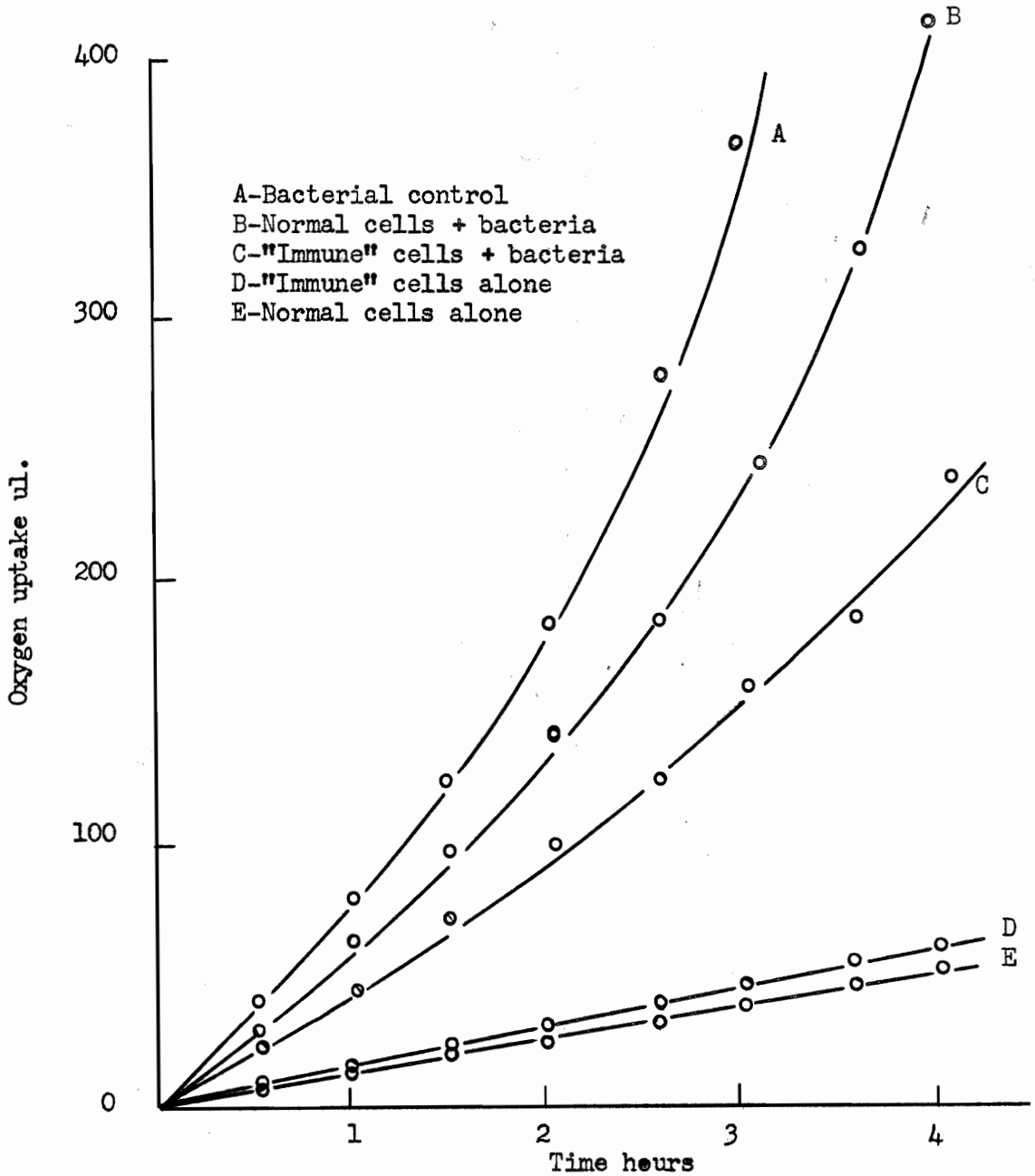

Figure 14

Oxygen uptake of S. typhimurium
incubated with normal and "immune" rabbit phagocytes

mice. These mice were challenged 12 hours later by intraperitoneal injection. Table XX demonstrates the effect of intraperitoneal injection of peritoneal phagocytes from immunized and normal (non-immunized) animals on survival of irradiated and nonirradiated mice challenged with K. pneumoniae. Under the experimental conditions it can be seen that in normal mice (group I and II) no difference can be demonstrated in the protective capacity of phagocytic cells from normal or immunized animals. Differences that may exist in these cells are possibly masked by the full complement of phagocytic cells possessed by the host. However, when mice were exposed to x-irradiation (450 r or an LD_{46}) and received injections of comparable numbers of normal or "immune" phagocytes (1.4×10^7 normal cells and 1.2×10^7 immune cells) on the 6th postirradiation day, when host defenses are depressed; it was found that "immune" phagocytic cells exhibit a greater capacity to reduce mortality than do normal phagocytic cells (compare group V and VI with group IV).

Figure 15 presents the results of an experiment which follows the same experimental design; however S. typhimurium was used as the challenge organism, and also as the immunizing agent for donor mice from which "immune" phagocytic cells were harvested. The radiation dose was 425 r or an LD_{26}. Mice received normal (2.02×10^7) immune cells (1.95×10^7) on the fifth postirradiation day and were challenged 12 hours later. It can be seen that challenge in the absence of phagocytic cells was rapidly fatal for all animals. Similarly, animals which received normal phagocytic cells all died. Immune phagocytic cells not only prolonged survival time of the animals that died, but significantly reduced mortality as measured by the Chi square technique. Attempts to

TABLE XX

EFFECT OF I.P. INJECTION OF PERITONEAL PHAGOCYTES FROM IMMUNIZED AND NORMAL ANIMALS ON SURVIVAL OF IRRADIATED AND NON-IRRADIATED MICE CHALLENGED WITH KLEBSIELLA PNEUMONIAE

Group	Treatment	Mortality %
I	Normal mice + "immune" cells + challenge	0
II	Normal mice + normal cells + challenge	0
III	Normal mice + saline + challenge	24(6/25)*
IV	X-ray + saline + challenge	81(13/16)
V	X-ray + "immune" cells + challenge	56(9/16)
VI	X-ray + normal cells + challenge	80(12/15)
VII	X-ray control (no challenge)	46(7/15)

*Mortality ratio

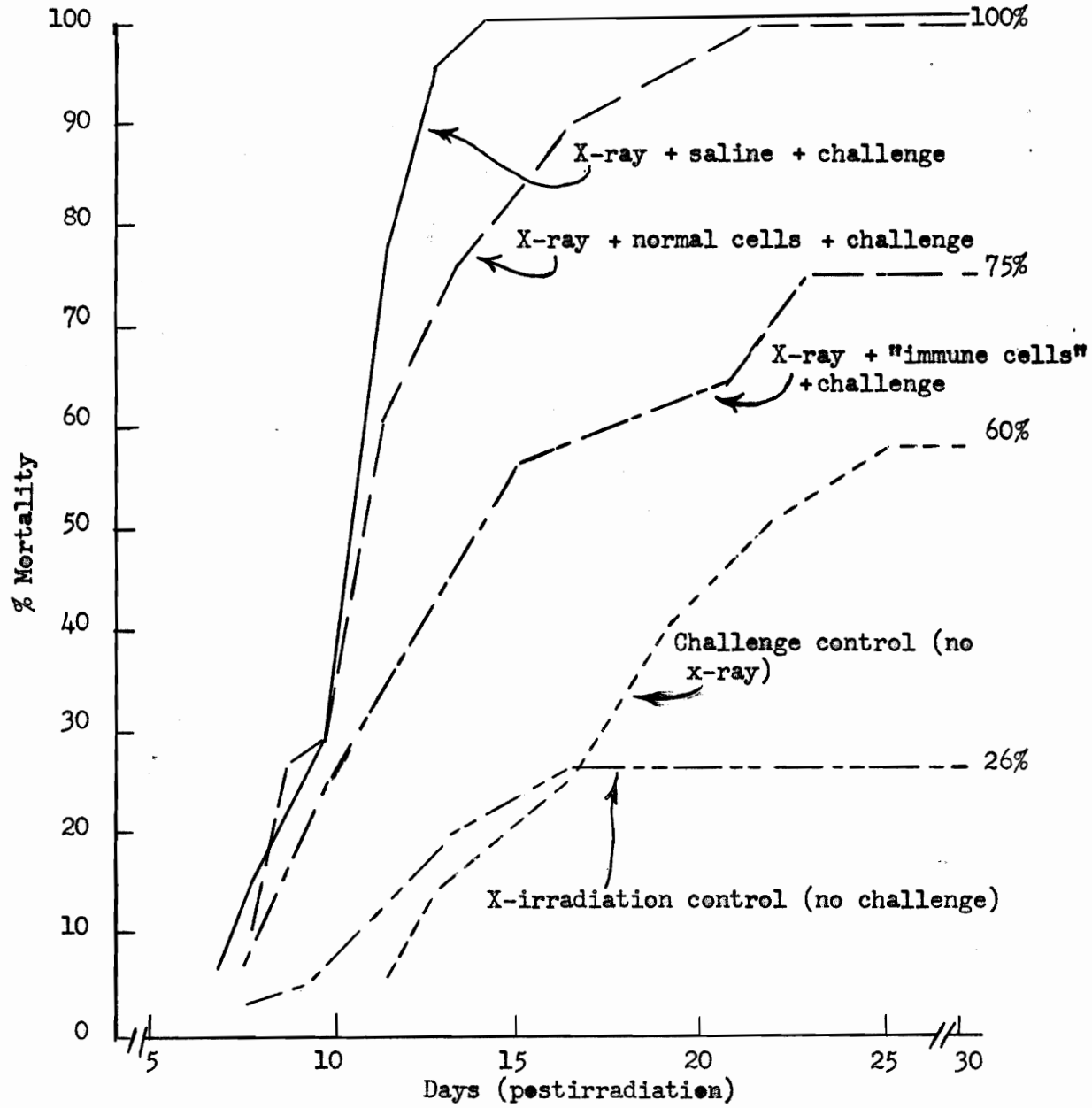

Figure 15

Protective effect of "immune" phagocytes injected i.p. prior to challenge with Salmonella typhimurium.

demonstrate intra or extra cellular antibody by agglutination techniques again were unsuccessful.

In an effort to demonstrate that this protection was due to the functional capacity of the donor cell, rather than enhancement of the animal's own cellular defenses due to mobilization or localization of cells at the site of challenge, "immune" and normal cells were collected from donor animals. These washed cellular suspensions were then divided into two equal volumes, cells in one volume were then disrupted by rapid freezing and thawing whereas cells of the second volume were left intact. Freezing was accomplished by use of a dry ice, acetone bath and thawing speeded by use of a 37°C water bath. This procedure was repeated 5 times after which the material was ground in a sterile mortar and pestle. Irradiated animals (425 r) received injections of intact or disrupted cell suspensions on the 6th postirradiation day, and were challenged 12 hours later with K. pneumoniae. It can be seen from Table XXI that intact "immune" cells significantly reduced mortality whereas the disrupted cellular suspensions failed in this capacity. (Compare group I and II with group V). Similar results can be seen using normal intact and disrupted cellular suspensions (Compare group III and IV with group V). This data might also be interpreted as evidence that intracellular non-agglutinating antibody did not aid in the observed differences in mortality following injection of "immune" and normal cells. However, as the physical means of disrupting cells (freezing and thawing) caused "clumping" of cellular protein, this method might have similarly altered or disterted antibody, thereby eliminating any protective value non-agglutinating antibody might have contributed, provided any such antibody was actually present.

TABLE XXI

LOSS OF PROTECTIVE EFFECT OF PERITONEAL

PHAGOCYTES FOLLOWING CELLULAR DISRUPTION

Group	Treatment	Mortality %
I	"Immune" cells + challenge	28%(7/25)*
II	Disrupted "immune" cell suspension +challenge	60%(15/25)
III	Normal cell + challenge	48%(12/25)
IV	Disrupted normal cell suspension +challenge	64%(16/25)
V	Saline + challenge	70%(17/23)
VI	X-ray control (no challenge)	20%(6/30)
VIII	Challenge control (no x-ray)	44%(11/25)

*Mortality ratio

Under the conditions of the experiments it has been demonstrated that phagocytic cells from animals actively immunized against bacterial agents of acute infection have an enhanced functional capacity independent of demonstrable antibody.

DISCUSSION

The experimental results herein reported have demonstrated that immunization can be used as a procedure for enhancement of defense mechanisms against infectious disease in x-irradiated animals. However, discussion of these results is necessary in order to understand the significance of this generalization.

In analyzing the observed failure to produce measurably increased resistance in rats, the possibility of "immunologic paralysis" was entertained because the K. pneumoniae organism used to prepare the vaccine had a large polysaccharide capsule. Felton (1949) observed that mice injected with relatively large amounts of pneumococcal polysaccharide are subsequently incapable of being immunized with the homologous antigen, and it was suggested that persisting antigen was adversely affecting cells responsible for antibody production. Dixon et al. (1955) have shown that tissue-fixed polysaccharide antigen is capable of combining with antibody and that the antibody is then promptly catabolized. These observations may account for the absence of circulating or protective antibody in the "immunologically paralyzed" animal. From such considerations it might appear attractive to suggest that in the present investigation a similar suppression of the immune response resulted from the administration of the capsular polysaccharide of K. pneumoniae. Such speculation, however, does not seem warranted because it is improbable that the dosage of antigen administered was sufficient to induce "paralysis"; furthermore, circulating antibody was present indicating that "paralysis" had not resulted from the immunizing procedure. An unanswered question remains; that is, did the

doses of the K. pneumoniae provide a maximum antigenic stimulus without introducing an excess of polysaccharide antigen which may have partially neutralized the antibody response?

Perhaps more productive speculation concerning the immunologic failure could be directed toward the ability of specific immunizing measures to increase the resistance of the rat above the inherently high level of natural resistance possessed by these animals. Pillemer et al. (1954) have found that the white rat is endowed with the highest serum properdin level among common laboratory animals and these workers suggest that this is an important defense mechanism for the rat. It is a classical observation that rats are less susceptible to postoperative infections than many other animals and that even if all aseptic precautions are disregarded most of the animals will remain free from infection (Ingle and Griffith, 1942). A high antibody titer may not play the dominant role in providing protection for the rat and may serve only as an ancillary mechanism to other native defenses of this animal. It is possible that antibody is not as significant as the cellular mechanism in protection against infection, a finding already reported for the albino mouse (Marcus and Donaldson, 1954).

From the data which have been presented, (immunization experiments with K. pneumoniae, S. typhimurium and B. tularense) it is evident that specific immunization prior to x-irradiation is a prophylactic measure which will reduce the mortality of mice to subsequently induced infection following radiation in doses approaching LD_{50} levels. Apparently a reciprocal relationship exists between the extent of the irradiation injury and the degree of resistance conferred. The greater the irradiation dose, the greater was the susceptibility to infection and the less was the protection offered by the immunization procedure.

In the present studies immunization with K. pneumoniae or S. typhimurium vaccine could not be expected to afford protection to mice exposed to high radiation levels, i.e., greater than an LD_{50}, because no measures were taken to control endogenous invasion by organisms of the normal enteric flora. In reality, therefore, two separate invasive forces contributed to the observed results: 1) the unrestricted advance in the x-irradiated animals of the normally avirulent enteric flora and 2) the superimposition of highly pathogenic organisms; the only attempts at control were pre-induced specific immunizing measures.

Taliaferro (1951) in analyzing the effect of x-ray on the resistance mechanisms suggested that demonstrable injury to the host by x-irradiation may be correlated with the presence of certain intermediate level of immunity. Below this level, if there is impairment of resistance mechanisms, no observable change will be noted in the course of the infection because the resistance is of such low level that no demonstrable protection is afforded. Above this level of resistance, even though impairment by x-irradiation may occur, there is still the necessary margin of reserve which will mask the injury. Evidence supporting this conclusion is demonstrated in Table IX where different methods were used in immunizing acutely and chronically irradiated mice, and in Table X where a greater resistance resulted from one immunizing procedure than from others, (compare groups 22a with 44a).

The data suggest that multiple mild exposures to x-irradiation (chronic exposure), even when extended over relatively long periods of time, did not increase susceptibility to infection or result in mortalities comparable to a single high exposure of irradiation. It is of interest to note that Blair's (1952) hypothesis relative to

chronic radiation exposure," . . . injury from penetrating radiation develops in proportion to the dose rate and is reapired spontaneously at a rate proportional to its magnitude except for an irreparable portion proportional to the total accumulated dose," which adequately accounts for impairment in mice if injury is measured by shortening of the life span, amy also be applicable, with slight modification, to the data presented here with respect to injury of host defense mechanisms. It can be suggested that with repeated mild exposure, if recovery of sensitive defenses is not complete, each successive radiation insult leaves an additional residuum of damage. The accumulation of such residual defects on certain critical cell systems of the host defense mechanisms would be made evident by increased susceptibility to infection and decreased efficiency of the host to combat invasions.

Reasoning from this hypothesis, one would not expect an increased susceptibility to infection with chronic radiation exposure until the injury imposed exceeded the rate at which efficient repair of damaged defenses takes place. Lorenz et al.,(1954) observed that although this chronic radiation exposure accelerated the death rate of exposed animals, it did not perceptibly lower the resistance of mice to infection. The workers suggested that probably some unknown systemic factor was responsible for the observed results. The hypotheses of Blair and of Lorenz et al. can be, to some extent, reconciled in the following hypothesis. A critical minimal radiation exposure threshold exists with relation to infection. Below this threshold no demonstrable damage to host defense mechanisms is present since a dynamic equilibrium between radiation injury and biologic repair is maintained. Under experimental

conditions this equilibrium can be destroyed by a variety of exposure patterns (i.e., by varying either the magnitude of exposures or the interval between successive exposures), thereby impairing the ability of the host to resist infection. On this basis one would not expect a lowered resistance to infection with chronic irradiation exposure until the injury imposed exceeded the rate at which repair of the damaged defenses takes place.

From the results presented on the effect of immunization and antibiotic therapy (Figure 6), it can be inferred that specific immunization and antibiotic therapy may be used with moderate success to increase the resistance of x-irradiated mice to infection. In the present investigation concomitant use of these measures prolonged survival time, but failed to alter mortality. It was speculated that by utilization of both these measures protection might be afforded to animals exposed to levels of x-irradiation greater than one LD_{50}. Therefore, animals were subjected to a total body dose of 425 r (LD_{64}). However, the challenge inoculum was of such magnitude that high mortality (LD_{52}) for nonirradiated (challenge control) animals resulted. Irradiated mice were completely overwhelmed and could not cope with the massive infections resulting from the challenging organisms. Had either a milder challenge infection or a lower radiation exposure level been used one would anticipate that host defenses enhanced by immunization and aided by the antibacterial action of antibiotics might suppress invasion and thereby reduce significantly the mortality of these animals. The magnitude of such protection can be predicted. That is, in the case of the parenterally challenged animal, any combination of irradiation exposure and challenge inoculum which exceeds an LD_{50}

for normal animals will, with high probability, prove fatal for immunized or immunized and antibiotic treated animals. The same prediction can be made for orally challenged animals. Although the data do not allow confident prediction in the case of respiratory challenge, one would suggest as a working hypothesis that the same prediction could be made for this situation.

In many types of systemic bacterial disease sublethal infection results in a high degree of active immunity.[y] It appears that this was not the case in the experiment with B. tularense (Table X). However, it should be kept in mind that the infection to confer immunity was induced with a relatively small number of organisms (16 or 162) in half the mice. Normal host defenses may have eliminated this number in such a rapid and efficient manner that an adequate antigenic stimulus did not occur. Therefore, in reality no immunity or only a very low-grade type of immunity may have been present in many of these animals. This might explain the failure to observe significant differences between groups 33a and 33b and between groups 44a and 44b (Table X).

The experiment which attempted to reduce mortality of irradiated mice by preradiation immunization with an antigenic extract of E. coli demonstrated that the mortality of mice exposed to $LD_{66.6}$ radiation could not be reduced by immunization against a given member of the normal intestinal flora. However, two factors should be considered which might well have doomed this experiment to failure: 1) No attempt was made to control the endogenous infection resulting from invasion of other enteric organisms. Therefore, equally rapid invasion by these organisms in both groups of mice were possible. Death from

these infections may have masked any protective effect that might have been afforded by the specific immunization procedure. 2) The feasibility of immunization with E. coli organisms is questionable. Demonstration of a specific antibody against pathogenic strains of E. coli has been reported in human volunteers only when large numbers of living organisms were injected. Attempts to show a rise in titer against a living "normal" strain was unsuccessful (Ferguson and Tune, 1952 and Neter et al., 1953). As the investigation was a pilot study, the experiment was carried out without regard to these inherent pitfalls for if significant differences had been obtained the desirability of a large scale study with multivalent vaccine prepared from offending members of the normal flora by the described method could not have been questioned.

If the prolonged survival time can be interpreted as suggestive evidence of protection, the preparation of multivalent vaccine from the members of the enteric flora most commonly responsible for blood stream invasion may still hold some promise as a possible prophylactic measure that might be utilized in conjunction with other therapy as a means for controlling postradiation morbidity.

Although the experimental results did not in every instance support the hypothesis that immunization is a significant protective measure, the foregoing discussion presents reasons for the occasionally observed failure; the characteristic trends are readily apparent and the validity of the conclusion drawn concerning the efficacy of immunization as a protective measure against the infectious component of the radiation syndrome in mice cannot be seriously questioned.

The hypothesis was suggested that if an interrelationship existed between antibody and bactericidal action of serum, or if serum

bactericidal action, although nonspecific, might be enhanced through active immunization, that quantitative diminution of bactericidal action following x-irradiation might be reversed by specific immunizing measures. Since the serum bactericidal activity was not increased by immunization, it is suggested that the bactericidal activity is maximum in the serum of the normal (non-immunized) rat. It should be noted that although immunization failed to enhance the bactericidal action of sera, that agglutinin titers were significantly increased (Tables XI and XII). Hence, evidence confirming the classical contention, that serum bactericidal action is non-antibody mediated is reported.

Furthermore, under the conditions of the experiments described, it seems probable that the loss of serum bactericidal action may not be a major factor contributing to the onset of bacteremia in either the irradiated rat or rabbit. These observations coupled with the knowledge that mouse serum has no significant bactericidal properties (Marcus *et al.*, 1954) induces skepticism concerning the comparative significance of this host defense mechanism in the protection of these species. Such speculation is based on the assumption that the loss of serum bactericidal activity *in vitro* reflects the similar capacity of serum *in vivo*. Such an extrapolation has little experimental foundation. Nevertheless, if *in vitro* studies are valid, the hypothesis that loss of serum bactericidal action results in the lymphogenous and hematogenous dissemination of infection receives no support from these experiments.

Properdin, a recently isolated serum protein, has been implicated as an essential factor in the observed bactericidal action of serum (Pillmer *et al.*, 1954). Serum properdin levels are markedly reduced following radiation exposure (Ross, 1956; Linder, 1957). It has

been suggested that decreased properdin levels result in postirradiation loss of serum bactericidal action (Ross, 1956). In view of the reported observations, this speculation concerning the mechanism of action of properdin in the defense of the host might likewise be questioned.

It is of interest to note that rabbits exposed to 600 r of x-irradiation failed to develop bacteremia. This is in agreement with the findings of Hammond and Miller (1955). These investigators found the incidence of bacteremia to be very low in rabbits exposed to 900 r of x-irradiation among animals in which 800 r was the LD_{50} and 1000 r the LD_{100}.

From the reported investigations the mechanism(s) responsible for the prevention of overwhelming bacteremia in rabbits and for its occurrence in rats and mice cannot be explained on the basis of loss of serum bactericidal action.

In considering the effect of x-irradiation on preformed antibody and its role in the protection of x-irradiated mice it may be well to recall that there is ample theoretical evidence to suggest a specific functional region in the protein molecule or molecular complex characterizing antibody (Pauling, 1940; Boyd, 1956 and Raffel, 1953). It is held, in current serologic theory, that it is this functional region that is responsible for interaction with antigen. Therefore, if inactivation of antibody is to take place, the action of ionizing radiation must, in some way (directly or indirectly) exert a destructive effect on this active grouping or configuration. One may speculate that oxidation-reduction reactions which are brought about by ionizing radiation might alter the functional activity of specific groups or that free radicals produced during the oxidation of adjacent reactants might exert an effect on the groups responsible for antibody specificity.

Furthermore, the breaking of a bond by the removal of a valence electron by a migrating positive charge might allow the splitting off of a prosthetic group or the molecular pattern might be disturbed with subsequent rearrangement resulting in a distorted configuration. Such speculation is analogous to that proposed for the deleterious effects or radiation on proteins, enzymes and other large molecules (Pollard, 1954).

If alteration occurred, the resulting destruction of antibody could be selective if the functional groups of various antibodies exhibited a greater sensitivity to x-irradiation. For example, the sensitivity of the sulfydryl groups to ionizing radiation has been clearly established, and if this group was implicated as one responsible for interaction with antigen in a particular antigen-antibody reaction, then the possibility of antibody being altered by ionizing radiation would be increased. Therefore, it could be concluded that the destruction of antibody would depend upon the sensitivity of the functional groups responsible for interaction.

The results reported here, that ionizing radiation had no effect on the titer of hemagglutinating antibody, may be taken as evidence suggesting that the functional groups responsible for interaction between antigen and antibody are not markedly altered. Barron (1954) has ventured the opinion "that nucleic acids are rather resistant to x-irradiation because they are well protected by other groups in the vicinity." Since the bulk of the molecule containing the antibody substance has nothing to do with the reactive groups, then antibody may be rather resistant to x-irradiation because the reactive groups are protected by other groups in the vicinity which react with the

free radicals produced during the oxidation of other reactants.

Whatever the mechanisms, results obtained (Figure 8) indicate that x-irradiation, even in highly lethal doses (LD_{100}), neither affected the titers of hemagglutinating antibody *in vitro*, nor altered the protective capacity of "immune" serums *in vivo* (Table XVI).

It has been demonstrated that x-irradiation reduced cellular response (Cronkite and Brecher, 1955) and suppresses such cellular functions as leukocyte migrations, (Shechmeister and Fishman, 1955) phagocytosis, (Taplin *et al.*, 1954) and intracellular digestion, (Donaldson *et al.*, 1956). Such depression has been found to be proportional to the irradiation dose employed.

The statement "immunological response is essentially antibody formation" (Haurowtiz, 1953) is widely accepted. The importance of cellular mechanisms in defense is often neglected. It seems certain that antibodies unite with the invading bacteria. The function of antibody in antibacterial immunity is usually tacitly assumed to be to serve as a preparation for the cellular destruction of the invading organisms by phagocytic cells. Available evidence suggests that antibodies are adjuvant factors of great importance in specific resistance. Nevertheless, even in many instances where humoral factors are of extreme importance, cellular destruction of bacteria is apparently as significant in resistance. In the present investigation this fact has been forcibly demonstrated. Little doubt is left here concerning the importance of the role played by antibody in the defense of the x-irradiated mouse. The observations (Figure 9) that antibody (serum from immune animals) was just as effective an agent in protection of mice which had been exposed to either 350 or 400 r of x-irradiation, but

failed completely at higher levels, indicates that for antibody to be effective in protection, cellular activity cannot be impaired. The hypothesis is suggested that even though phagocytic cells of actively immunized animals may be endowed, as a result of the immunizing procedure, with certain enhanced intrinsic properties important in host defense which they lack under normal conditions, that this may be secondary to the role played by antibody when adequate numbers of cells are present. This may be the case in the reported study (Figure 9) in normal (nonirradiated) animals or in animals which had received a total body dose of 350 or 400 r of x-irradiation. However, at a time when cell populations are critically reduced or functionally impaired, i.e., at the 425 r level (Figure 9), an enhanced capacity of the remaining cells to effectively destroy invading bacteria may be a factor of importance in the successful defense of the host. For this reason, it is suggested that active immunization was more effective in reducing mortality than was passive immunization. At higher levels, i.e., 450 r, cellular response had been so impaired that even enhanced functioning of the remaining cells may have made successful defense impossible. Therefore, immunization (either active or passive) did not afford protection to the animals.

Since x-irradiation had no demonstrable effect on preformed antibody, this important defense mechanism apparently remained unaltered, actively participating in the defense of the irradiated host. The hypothesis is suggested that the increased susceptibility to infectious disease seen in x-irradiated animals may be attributed primarily to the depression of other most likely cellular, host defense mechanisms.

The observation that preformed antibody was not affected following x-irradiation exposure and the reported finding that x-irradiation suppresses phagocytosis and intracellular digestion (Donaldson and Marcus, 1956) implies that the development of infections in irradiated animals is dependent upon the number of functional active phagocytes remaining uninjured following x-irradiation exposure. This suggestion received support in the present investigation where mobilization and localization of leukocytes at the site of challenge enhanced the resistance of the host.

Furthermore, Hollingsworth and Finch (1956) have reported that transfused leukocytes significantly lowered bacteremia in x-irradiated rats. Similarly Congdon et al. (1956) noted that "leukomoid" (sic) blood from tumor bearing mice promoted recovery of total body irradiated mice as measured by survival and regeneration of haematopoietic tissue. Therefore, it was thought that injection of functionally active leukocytes following exposure to ionizing radiation might be of value in controlling the infections that develop following irradiation injury.

The present experimental data presents evidence that injection of leukocytes may increase survival of whole body irradiated mice. It has been conclusively demonstrated that administration of haematopoietic tissue preparations (suspensions of bone marrow or splenic tissue) can modify the lethal effects of whole body ionizing x-irradiation (Lorenz et al., 1951; Jacobson et al., 1951; Silverman et al., 1956 and Trenton, 1956). It may be deduced that the function of shielded marrow or injection of haematopoietic tissue is to carry on haematopoieses and provide functional activity during the postirradiation period when the host's marrow is inactive. This "tiding over" period is necessary until resumption of activity be damaged tissue takes place. At radiation

levels where complete destruction of haemapoietic tissue ensues, total repopulation by donor cells results. Since marrow therapy must be instituted immediately following exposure to x-irradiation if protection is to result, it appears that a period of differentiation and maturation is necessary for the development of essential cellular elements. It has been shown that the primitive marrow cells are totipotential and give rise under proper stimulus to any type blood cells. It is postulated that disorders of leukopoieses, which follow x-irradiation may be more important than disorders of erythropoiesis and that, in response to increased demands for leukocytes (phagocytic cells) immediately following irradiation, that increased differentiation along these cell lines results. Gilbert et al. (1957) have shown that x-irradiation has no effect on the red cells in circulation or on their life span although erythropoieses may be completely inhibited. Although anemia results, this may be secondary to the immediate effects which result from severe leukopenia.

From the present investigation, it is suggested that the procedure of injection of peritoneal phagocytes represents a substitution of essential cellular elements that are no longer being produced. The importance of infused leukocytic cells may reside in that they provide an artificial phagocytic system necessary for maintenance of life processes until partial regeneration and recovery of damaged marrow has taken place. Maintenance of phagocytic function is essential for survival of x-irradiated animals. Phagocytosis is necessary to effectively cope with infections, usually of endogenous origin, which occur in these animals. Furthermore, it is by this process of phagocytosis that normal physiological processes in the tissues are maintained by

the neutralization or destruction of noxious substances of endogenous origin (Bloom's "physiological inflammation") released by injured and degenerating cells following irradiation.

Although in theory, by injection of actively phagocytic cells, one should be able to conduct definitive experiments to determine the role played by phagocytic cells in the protection of the irradiated host, complicating factors inherent in the biological system increase in magnitude with a single and each succeeding injection of these cells. That is, in an effort to establish a more effective clearing mechanism by injection of relatively large numbers of phagocytic cells, one creates a further shift from homeostasis by increasing the load to be cleared by the host's already overtaxed and declining phagocytic system. This shift occurs because the life span of the donor cells is short in comparison with the length of time that their function is needed. That is, before adequate regeneration and recovery of function of the host haematopoietic system occurs, death and dissolution of these donor cells yield toxic products and cellular debris to be removed if homeostasis is to be maintained. Therefore experiments are difficult to design that can yield definitive information concerning the relationship among such complicating factors as the number of donor cells, the time of injection, the route, and the number of injections necessary.

Gyi (1957) has reported that x-irradiation in doses of 350 or 450 r in mice significantly depressed the intracellular digestion of chicken erythrocytes by peritoneal phagocytes by the sixth postirradiation day. Similar observations were obtained when rabbits were exposed to 600 r of x-irradiation and cytopeptic activity was determined

on the seventh postirradiation day. Immunization with chicken erythrocytes caused an increase in the digestion of these cells by the phagocytes of mice and rabbits. However, it was reported that the immunization-induced increase in digestion was reversed by x-irradiation.

In the present studies the observations that injection of "immune" phagocytes decreased mortality may be related to an increased intracellular digestive capacity of the injected phagocytes following immunization with K. pneumoniae or S. typhimurium.

Observations similar to those reported in this investigation, that is, that immunization results in the enhanced ability of phagocytes to destroy or inhibit multiplication of viable organisms and that this is not dependent on humoral factors, have been reported in the literature review of this thesis. It should be recalled that Lurie (1942), Suter (1953) and Raffel (1955) have found inhibition of multiplication of tubercle bacilli in macrophages obtained from immunized animals. A similar inhibitory effect has been reported by Pomales_Lebron and Stinebring (1957) with macrophages obtained from animals immunized against B. abortus. From these reported observations and those presented in this investigation, it is apparent that evidence is accumulating to suggest strongly that immunization induces significant changes in the phagocytic cells. Since, in the present investigation, this capacity could be demonstrated in the living animal only when the cell population of the host was critically reduced, it is suggested that this enhanced functional capacity, although contributing to heightened resistance, may be secondary to the role of humoral antibody in many acute infections. Nevertheless, in those instances where antibacterial resistance cannot be correlated with antibody titer, the superior functional capacity of phagocytic cells may play the dominant role in the host's defense.

SUMMARY

It has been found that immunization with K. pneumoniae prior to x-irradiation, increased the resistance of mice to challenge with an aerosol of virulent organisms of this species. It was noted that as the radiation dose levels were increased, there was a progressive fall in the resistance of both normal and immunized animals. The greater the radiation dose the greater was the susceptibility to infection, and the less was the protection offered by the immunization procedure. However, this protective effect of preradiation immunization was not destroyed at sublethal to midlethal levels of exposure, i.e., levels at which mortality resulting from overwhelming infections are implicated as an important cause of death.

Subsequent studies showed that active immunization prior to x-irradiation increased the resistance of mice to infection via the oral route with S. typhimurium or B. tularense. This protective capacity was noted at all levels of irradiation below an approximate LD_{50} but was destroyed if higher irradiation doses were administered.

In these experiments it was found that chronic exposure (to 900 r) given in regular small increments was far less detrimental to host defenses than smaller lethal doses acutely administered. The hypothesis has been enunciated that a critical minimal radiation exposure threshold exists with relation to infection and route of infection. Below this threshold no demonstrable damage to the host defense mechanisms is present, since a dynamic equilibrium between radiation injury and biological repair is maintained. This equilibrium can be destroyed by excessive irradiation doses thereby impairing the ability of the host to resist infection.

If antibiotic therapy (penicillin, 5000 units and streptomycin, 5 mg) was utilized in conjunction with specific immunization the ability to protect mice was greater than when either of these measures were used independently. The mean time of death for untreated orally challenged mice was 9.32 days postirradiation; this was extended to 10.48 days for immunized mice, to 11.12 days for antibiotic treated mice, and to 13.6 days when both measures were utilized.

Immunization of mice with a vaccine prepared against two strains of E. coli isolated from the blood of lethally radiated mice failed to enhance resistance against normal endogenous invasion resulting from radiation (LD_{66}) injury as measured by 30 day mortalities. Immunization, however, slightly prolonged the survival time of these animals. If this data can be interpreted as suggestive evidence of protection, the preparation of multivalent vaccines against the offending flora might prove useful in controlling invasion by the normally non-pathogenic enteric organisms following radiation injury.

Experiments designed to determine the effect specific immunization might have upon the bactericidal activity of sera from normal and x-irradiated rats demonstrated that immunization did not enhance the bactericidal activity of these sera and in no way reversed the depressed bactericidal action of sera following irradiation exposure. Futhermore, investigations to determine if the depression of serum bactericidal activity can be temporally related to, or is merely coincident with the onset of bacteremia in the irradiated animal revealed no correlation between the onset of bacteremia to decreased serum bactericidal activity. The hypothesis that loss of serum bactericidal activity results in the lymphogenous and hematogenous dissemination of infection received no support from these experiments.

It was found that induced agglutinin titers for human type A erythrocytes were not decreased by a 300, 500 or 700 r total body dose of x-irradiation 3, 7, 11 or 15 days following irradiation exposure in albino rats. Sera from mice immunized against K. pneumoniae and then subjected to lethal irradiation was just as effective in passive immunization for prevention of death in recipient animals as was sera from immunized non-irradiated mice. Passive immunization of normal (nonirradiated) mice and mice which had received low levels of irradiation (300 or 400 r) proved just as effective a measure for protection as did active immunization. At higher levels antibody (passive immunization) afforded no protection whereas active immunization enhanced resistance.

It was observed that a cellular infiltration of phagocytic cells induced in the peritoneum of x-irradiated mice by injection of a 0.1% glycogen saline solution enhanced the resistance of these animals to a later challenge infection with K. pneumoniae. A single intraperitoneal injection of heterologous leukocytes (rat or rabbit) administered on any given day in the first postirradiation week failed to significantly reduce mortality of non-challenged irradiated mice. However animals which received two intraperitoneal injections of homologous phagocytic leukocytes at critical postirradiation periods exhibited reduced mortalities.

Investigations carried out in vitro demonstrated that phagocytic cells of actively immunized animals had an enhanced ability to destroy or inhibit multiplication of viable organisms independent of demonstrable agglutinating antibody. Similarly in vivo experiments demonstrated that phagocytic cells of actively immunized animals had enhanced phagocytic

function in that injections of these cells enhanced the resistance of irradiated mice to subsequent challenge with the homologous organisms whereas cells obtained from normal mice afforded no protection.

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THE EFFECT OF IMMUNIZATION ON DEFENSE MECHANISMS AGAINST INFECTIOUS DISEASE IN IRRADIATED ANIMALS

by

Eugene Hafen Perkins

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It has been found that immunization with K. pneumoniae prior to x-irradiation, increased the resistance of mice to challenge with an aerosol of virulent organisms of this species. Subsequent studies showed that active immunization prior to x-irradiation increased the resistance of mice to infection via the oral route with S. typhimurium or B. tularense. It was noted that as the radiation dose levels were increased, there was a progressive fall in the resistance of both normal and immunized animals. The greater the radiation dose the greater was the susceptibility to infection, and the less was the protection offered by the immunization procedure. However, this protective effect of preradiation immunization was not destroyed at sublethal to near midlethal levels of exposure, i.e., levels at which mortality resulting from overwhelming infections are implicated as an important cause of death.

In these experiments it was found that chronic exposure (to 900 r) given in regular small increments was far less detrimental to host defenses than smaller lethal doses acutely administered. The hypothesis has been enunciated that a critical minimal radiation exposure threshold exists with relation to infection. Below this threshold no demonstrable damage to the host defense mechanisms is present, since a dynamic equilibrium between radiation injury and biological repair is maintained. This equilibrium can be destroyed by excessive irradiation doses thereby impairing the ability of the host to resist infection.

If antibiotic therapy (penicillin, 5000 units and streptomycin, 5 mg) was utilized in conjunction with specific immunization the ability to protect mice was greater than when either of these measures were used independently.

Immunization of mice with a vaccine prepared against two strains of E. coli isolated from the blood of lethally radiated mice failed to enhance resistance against normal endogenous invasion resulting from radiation (LD_{66}) injury as measured by 30 day mortalities.

Experiments designed to determine the effect specific immunization might have upon the bactericidal activity of sera from normal and x-irradiated rats demonstrated that immunization did not enhance the bactericidal activity of these sera and in no way reversed the depressed bactericidal action of sera following irradiation exposure. Futhermore investigations to determine if the depression of serum bactericidal activity can be temporally related to, or is merely coincident with the onset of bacteremia in the irradiated animal revealed no correlation between the onset of bacteremia to decreased serum bactericidal activity.

It was found that induced agglutinin titers for human type A erythrocytes were not decreased by x-irradiation 3, 7, 11 or 15 days following irradiation exposure in albino rats. Sera from mice immunized against K. pneumoniae and then subjected to lethal irradiation was just as effective in passive immunization for prevention of death in recipient animals as was sera from immunized non-irradiated mice. Passive immunization of mice which had received low levels of irradiation (300 or 400 r) proved just as effective a measure for protection as did active immunization. At higher levels antibody (passive immunization) afforded no protection whereas active immunization enhanced resistance.

It was observed that a cellular infiltration of phagocytic cells induced in the peritoneum of x-irradiated mice by injection of a

glycogen saline solution enhanced the resistance of these animals to a later challenge infection with K. pneumoniae. A single intraperitoneal injection of heterologous leukocytes administered on any given day in the first postirradiation week failed to significantly reduce mortality of non-challenged irradiated mice. However, animals which received two intraperitoneal injections of homologous phagocytic leukocytes at critical postirradiation periods exhibited reduced mortalities.

Investigations carried out in vitro and in vivo demonstrated that phagocytic cells of actively immunized animals had an enhanced phagocytic function independent of demonstrable agglutinating antibody.

RESEARCH PROPOSALS

submitted

by

Eugene Hafen Perkins

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RESEARCH PROPOSALS

1. A characteristic finding in mice which have suffered radiation injury is the development of overwhelming bacteremia. It is hypothesized that onset of bacteremia can be related to impairment of phagocytic function which normally effects the removal of bacteria from the circulation.
2. An investigation will be carried out to determine if the lack of correlation, often observed, between circulating antibody titers and resistance to infection results from a failure to evaluate the contribution of non-precipitating or nonagglutinating protective antibody.
3. The rejection of a homograft, according to current theory, is related to the formation of humoral antibodies directed against the tissue of the graft. It is proposed that formation of antibodies by the graft and directed against the host may be of equal significance in the observed late deaths of x-irradiated animals receiving heterologous bone marrow.
4. An investigation to determine the mechanism of action of adjuvants in promoting antibody formation will be carried out. The hypothesis is enunciated that although the value of a given adjuvant may be related to retarded absorption, destruction and elimination of antigen that a correlation exists between the effectiveness of the adjuvant and its ability to quantitatively elicit a specific cellular inflamatory response.
5. Difference in the native resistance of species can be correlated with the phagocytic and intracellular digestive (cytopeptic) capacities of reticulo-endothelial cells.

6. It is suggested that peak antibody titer may not be as reliable a criterion for measurement of host resistance against infectious disease as is the combined observations of rate of appearance of circulating antibody, the mean titer, the duration of antibody response and the rapidity of decline of antibody titer. Therefore, efforts to investigate this hypothesis should be carried out.
7. An investigation is proposed to determine the cytopeptic index in patients with hypogammaglobulinemia and to relate this to resistance.
8. An investigation to determine if properdin will increase phagocytic or cytopeptic action in vitro will be conducted, in order to assess the role properdin may play in mice where the action of properdin cannot be attributed to bactericidal activity.
9. Two serum bactericidal mechanisms exist, one inactivated at 55°C and the other stable at this temperature. An investigation to elucidate the serum components other than complement which result in serum bactericidal activity and to determine if a relationship exists between the two systems is proposed.
10. The generalization is made that all animals possess complement. However, it is reported that certain species lack one or more components of complement. These observations may not be correct and it is hypothesized that the present classical assay reagents (SRBC and rabbit amboceptor) may be unsuitable for demonstration of complement components in all species.